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Antioxidant Candidates in Deep Eutectic Solvent Extract of Choline Chloride-Oxalic Acid from Sago Bark (Metroxylon sagu L. Rottb)

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Abstract

The waste deposit from sago starch extraction (*Metroxylon sagu* L. Rottb.) from sago trunks in Palopo City, Luwu Regency, North Luwu Regency and East Luwu Regency is quite piled up. Many studies on the utilization of liquid waste from the sago starch production process have been published. Meanwhile, the utilization of solid waste has not been widely carried out. Identification of liquid extract compounds from sago bark waste with environmentally friendly solvents such as deep eutectic solvent (DES) is still minimal. This study aims to identify the potential of DES extract compounds from sago solid waste. The DES used is DES Choline Chloride-Oxalic Acid with a mole ratio of 1:2. Extraction was carried out using the sonication method for 30 minutes, 50 W, room temperature, with a ratio of 80 mesh sago bark to DES of 1:10 (g/ml). The extract was filtered with 2.5 µm pore paper. Screening of compounds in the filtrate using GC-MS. DES was successfully synthesized while still showing the main functional groups according to the IR spectrum. GC-MS analysis of the filtrate showed that there were 8.36% squalene-like compounds; 5.37% n-hexadecanoic acid-like compounds and the content of other compounds besides solvents was ≤1%.

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Introduction

Sago waste is the largest agricultural waste found in Luwu Regency, North Luwu Regency, East Luwu Regency and Palopo City. Sago waste comes from the extraction of sago starch flour deposited on sago trunks. From this activity, the waste produced consists of 17% solid waste and around 80% liquid waste (Chua et al., 2021). In the last 5 years, the latest studies on the use of sago liquid waste include as a culture medium for Chlorella sp. (Effendi et al., 2023) and as a component of non-microplastic microbeads with chitosan which has antimicrobial properties (Silviana et al., 2022). Meanwhile, sago solid waste is more familiar as a basic ingredient for liquid organic fertilizer (Rosalina & Febriadi, 2019), and alternative animal feed (Siswati et al., 2023). The utilization of solid sago waste by relying on its compound components is still limited, one of which is as a potential source of bioethanol fuel (Numberi, 2022) and its potential as a briquette made from a mixture of coconut skin (Afna et al., 2021). Solid sago waste contains around 42% lignin (Lestari et al., 2022). Lignin is a natural polymer whose presence is always found together with cellulose and hemicellulose in plant biomass (Cao et al., 2019). The difference is that lignin is more polar than cellulose and hemicellulose, so polar solvents will be more suitable for extracting lignin from plant biomass (Zoghlami & Paës, 2019). Cellulose and hemicellulose have predictable structures, but the structure of lignin is quite complex (Ma et al., 2021) and is highly dependent on the source and age of the plant biomass, the type of soil and geology of the plant biomass source and the extraction procedure (Komisarz et al., 2023). This also causes the monolignol composition of lignin to differ from each plant biomass (Ruwoldt et al., 2023). In the last 3 years, research examining the chemical components of sago bark has led to diversity (Dinata et al., 2023) and components of sago solid waste biomass and the chemical content and characteristics of sago (Istikowati et al., 2023).

This study focuses on the identification of lignin-like compounds or cellulose or hemicellulose in sago bark waste extracted green using deep eutectic solvent (DES) oxaline (Choline Chloride-Oxalic Acid). Sago bark waste is a type of waste resulting from the extraction of sago starch from sago trees that has not been widely studied. In this study, the content of compounds in sago bark waste was studied through extraction with sonication and DES solvent. Various methods of lignin extraction in plant biomass have been widely used. The lignin extraction method through alkali hydrolysis produces 9% lignin klason and 4% dissolved lignin with a rough surface and high purity. Lignin extraction through the glycerolysis process obtains 12.5% lignin klason containing 3.5% dissolved lignin (Hassan & Badri, 2014). Lignin extraction from Alfalfa grass using formic acid/acetic acid produces 34% lignin, but requires bleaching with H_2O_2 at the purification stage (Watkins et al., 2015). With the development of more environmentally friendly extraction methods, lignin extraction methods from plant waste have also increased. One of them is by utilizing a sonicator to obtain nanoparticle lignin from wheat straw and Sarkanda grass biomass. However, the type of solvent used is not explained in detail (Gilca et al., 2015). DES solvent is a solvent made by mixing a hydrogen bond donor compound with a hydrogen bond acceptor compound. Its properties are similar to ionic liquids, easy to synthesize, and easy to decompose into its constituent components. The extraction method uses a sonication technique which is considered to be able to increase extraction efficiency by damaging cells and accelerating the dispersion of the extract into the solvent (Pham et al., 2022; Xiaokang et al., 2020). So far, there has been no in-depth study discussing the deep eutectic solvent oxaline extract compound from sago bark waste. The results of the identification of extract compounds are expected to be used as additional references in further studies of sago solid waste extraction with specific compound targets, as well as the utilization of compounds in the extract that have been successfully identified.

Method

The research sample was the outermost sago bark with a thickness of ±3 cm which had just had its starch removed and was air-dried at room temperature. The dried sago bark was then ground and sieved until it passed 80 mesh.

Deep Eutectic Solvent (DES) choline chloride-oxalic acid was made with a mole variation of 1:2 by mixing 5.560 g of choline chloride and 3.601 g of oxalic acid in a 100 mL Durham bottle. The mixture was heated at 61°C while stirring at 350 rpm. From this stage, a clear single-phase liquid was obtained which was then confirmed for its interaction via FT-IR. The FT-IR spectroscopy used was IR Prestidge 21 Shimadzu.

Extraction of compounds in sago bark was carried out at room temperature by mixing about 1 g of sago bark powder with 10 mL of DES choline chloride-oxalic acid. The mixture was sonicated for 5 minutes with an ultrasonic bath at a frequency of 40 KHz 50 W. Then, the mixture was vacuum filtered using 2.5 μm pore size filter paper. The filtrate was analyzed using GC-MS 2010 Ultra Shimadzu.

Results and Discussion

1. Synthesis of Deep Eutectic Solvent (DES) Choline Chloride – Oxalic Acid

DES which was made by mixing the two component compounds was successfully synthesized as shown by the appearance of the mixture being in one phase. The DES made was confirmed through the interaction of the components in the successfully extracted compounds. The interaction of the components in question can be seen in the interaction pattern of functional groups.

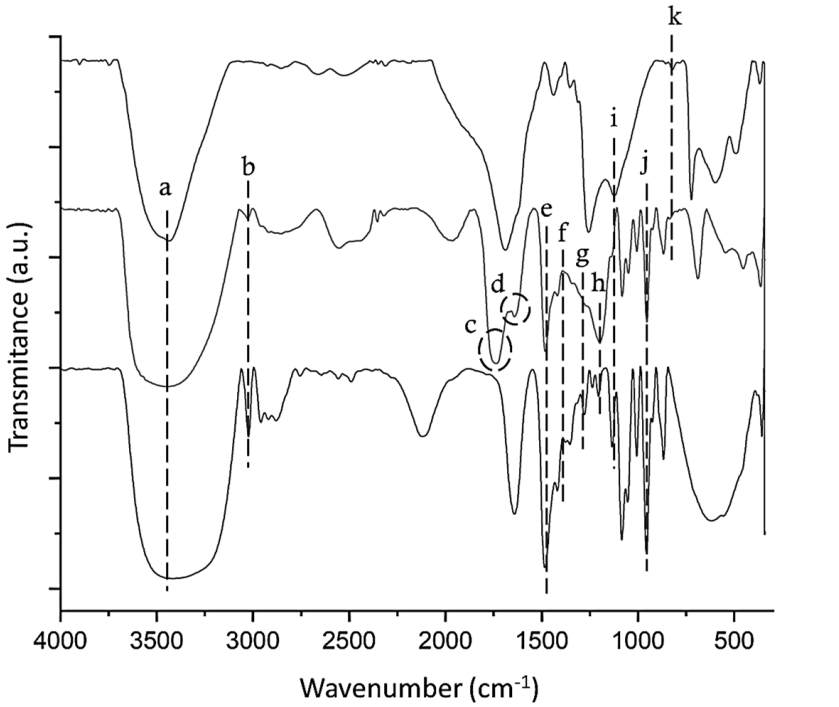

Figure 1. IR spectra of Oxalic Acid (top), DES Choline Chloride–Oxalic Acid (middle) and Choline Chloride (bottom)

Table 1. Wavenumber details of IR absorption in DES and its components

Wavenumber (cm^{-1})			Assignments	Notation on Figure 1
Choline Chloride	DES	Oxalic Acid		
3415	3441	3433	Stretching vibration O-H of H_2O	a
3024	3026		Stretching vibration CH_3	b
	1732		(doublet peak) Stretching vibration C=O	c
		1687	Stretching conjugated C=O (dimer)	
	1641		(doublet peak) bending vibration N	d
1483	1481		Bending vibration CH_2	e
1419	1417		Bending vibration C-O-H	F
1354	1334		Stretching vibration C-N	g
1278	1278		Bending vibration C-O-H	h
		1257	Stretching vibration C-O (dimer)	
1205	1199	1122	Stretching vibration C-O	i
1006	1006		Stretching vibration C-O	j
	954	931	*out of plane* vibration O-H	k
		723	Bending vibration C=O (Muthuselvi et al., 2016)	

Details of IR absorption in each spectrum in Figure 1 are presented in Table 1. Based on Figure 1 and Table 1, it is known that there are absorption numbers that shift from the choline chloride spectrum and the oxalic acid spectrum but still appear in the DES spectrum. These vibrations include the O–H

stretching vibration of water molecules (3415 cm^{-1} in choline chloride, 3433 cm^{-1} in oxalic acid and 3441 cm^{-1} in DES). And the C–O stretching vibration (in choline chloride at 1205 cm^{-1}, 1122 cm^{-1} in oxalic acid and at 1199 cm^{-1} in DES). While the IR absorption in the DES spectrum that is similar to that in the Choline Chloride spectrum includes the CH_3 stretching vibration (in choline chloride at 3024 cm^{-1} and in DES at 3026 cm^{-1}). There is also a similarity in the CH_2 stretching vibration (wavenumber 1483 cm^{-1} in the choline chloride spectrum while in DES it is found at number 1481 cm^{-1}). The C–O–H bending vibration of choline chloride (1419 cm^{-1}) also shifts in the DES spectrum (1417 cm^{-1}). In addition, similarities in the absorption numbers in the C–O stretching vibration in the choline chloride spectrum and the DES spectrum (1006 cm^{-1}) are also observed. While the IR absorption of the DES spectrum that is similar to the IR absorption of the oxalic acid spectrum is only the O–H out of plane vibration (the oxalic acid spectrum shows absorption at number 931 cm^{-1} while in the DES spectrum it is at number 954 cm^{-1}). However, there are 2 new absorption peaks in the DES spectrum (notation c and d in Figure 1) which are shown at numbers 1732 cm^{-1} and 1641 cm^{-1}. These absorption peaks are consecutive double peaks as a partial overlapping interaction between the C=O functional group and the N–H functional group (Pavia et al., 2009). This indicates that DES has been successfully synthesized as evidenced by the presence of each IR absorption from choline chloride and oxalic acid in the DES spectrum.

2. Sago Bark Extraction

The sample in this study was the outer part of the sago bark from which the starch had just been removed, dried and then finely sieved to increase the surface area during extraction. The extraction process was carried out using the sonication method at a low frequency of 50 W. Next, the compounds in the filtrate obtained from the extraction were screened. Based on Figure 2, which is the chromatogram of the DES filtrate, it is known that 102 compounds were identified. The sloping filtrate chromatogram confirms that the sample cell walls are almost completely damaged by the sonication treatment. Among these compounds, there are several peaks that have the highest intensity as shown in Table 2.

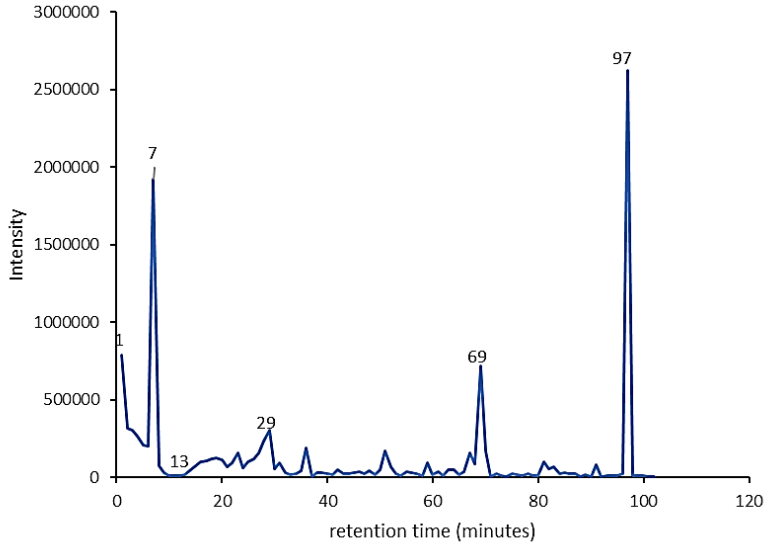

Figure 2. Filtrate Chromatogram of sago bark DES extract

Table 2. Compound with highest intensity in the chromatogram

Peak	Compound name	Information
1	Ethanol, 2-(dimethylamino)-(CAS)	GC solvents
7	Propanoic acid	
13	Choline Chloride	DES component
29	Ethanol, 2-(dimethylamino)-(CAS)	GC solvents
69	n-Hexadecanoic acid	Antioxidant candidate (Idu et al., 2021)
97	Squalene	Antioxidant candidate (Correia et al., 2022)

Data in table 2 shows that the compounds that make up the DES solvent used in the extraction stage do not react with the compounds contained in sago bark. Hexadecanoic acid compounds are compounds that are often found in organic extracts of plant essential oils (Idu et al., 2021). Meanwhile, the compound with peak number 97 as shown in Figure 2 is squalene. Squalene is a terpenoid compound that is widely found in animal cells (Budge & Barry, 2019) and plant cells (Correia et al., 2022). And this finding is different from the study which states that solid waste from sago processing produces lignin (Lestari et al., 2022). We suspect that cell damage caused by sonication treatment and the acidity level of DES Choline Chloride-Oxalic Acid (pH = 1) causes the decomposition of the lignin structure as an aromatic compound entity that is widely found in cambium plants (Majda, 2022). The decomposition of the lignin structure is thought to initiate the formation of squalene and simple acids such as propanoic acid (Wang et al., 2022; Wufuer et al., 2021) which are shown in Table 2. The decomposition of the aromatic structure of lignin causes the formation of long-chain unsaturated compounds such as squalene which have the potential to be used in the induction of cancer cell death (Bhat et al., 2023).

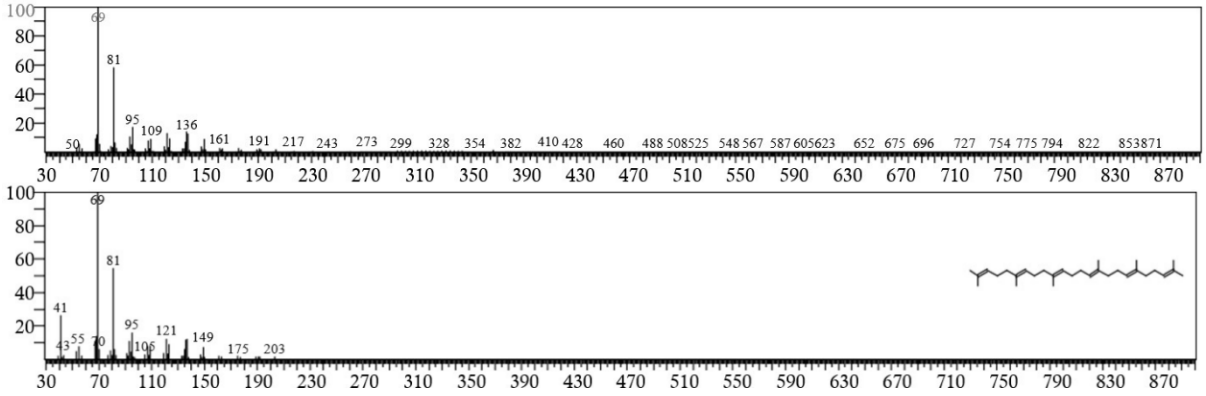

Figure 3. Comparison of mass spectrum graphs of filtrate (top) and squalene standard (bottom)

Figure 3 presents a graph comparison of the mass spectrum between squalene-like compounds in the filtrate with the mass spectrum of standard squalene. Based on this, the peaks in the mass spectrum of the filtrate are more than those in the mass spectrum of the standard. In addition, from a number of peaks found in both mass spectra, there are 3 similar peaks. Similar peaks in both mass spectra include the highest peak at 69 amu as the base peak, the peak at 81 amu and the peak at 95 amu. Other peaks with minimal heights in the mass spectrum of the filtrate (161 amu, 191 amu, 217 amu, 243 amu, 273 amu, 299 amu and 328 amu) and in the mass spectrum of standard squalene (43 amu, 175 amu and 203 amu) have not been explained and are thought to be lines that appear as a result of the loss of one or more hydrogen atoms during the fragmentation process in the evaporation of the sample through the ionization chamber of the device. Meanwhile, other unidentified peaks with quite good heights indicate that in the structure of the compound in the filtrate that is similar to squalene, there are more other carbon atoms whose structure has not been identified. We suggest further studies that could accommodate the identification of the structure of squalene-like compounds in the filtrate.

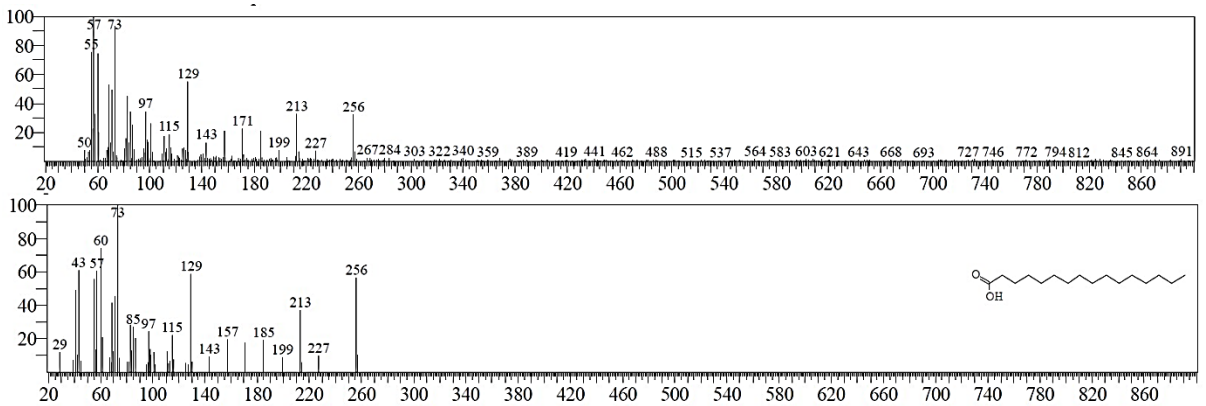

Figure 4. Graphs comparison of the mass spectrum of the filtrate (top) with the mass spectrum of n-hexadecanoic acid standard (bottom)

The hexadecanoic acid identified in the GCMS results (peak 69 in Figure 2 and in Table 2) is another candidate antioxidant compound identified in the filtrate. The comparison mass spectrum of the filtrate with the standard mass spectrum of hexadecanoic acid is shown in Figure 4. As shown, it is known that there are 7 similar peaks. The peaks include 73 amu as the base peak, 97 amu, 129 amu, 143 amu, 199 amu, 213 amu and 256 amu. As with the mass spectrum of the filtrate compared with the standard mass spectrum of squalene, the mass spectrum of the filtrate compared with the standard mass spectrum of hexadecanoic acid also has more peaks. This means that the filtrate containing compounds similar to hexadecanoic acid has other compounds that have not been identified by GC-MS. Structure elucidation via H-NMR together with C-NMR combined with NMR HSQC is feasible to ensure some antioxidant structures in the filtrate. With peaks that are more similar to the hexadecanoic acid standard, it is known that the level of similarity of the compounds in the filtrate to hexadecanoic acid is quite high.

Conclusion

The use of DES Choline Chloride-Oxalic Acid as a solvent in sonication extraction for sago bark samples (*Metroxylon sagu* L. Rottb.) increases the potential of the sample as an alternative source for antioxidant candidate compounds such as hexadecanoic acid and squalene. Sonication of 50W for 30 minutes on the sample was able to break down cell walls and produce 102 compounds in the extract. DES solvent at low pH is considered capable of breaking down lignin compounds from the sample into unsaturated compounds and simple organic acids such as propanoic acid without reacting to produce new compounds. The concentrations of antioxidant candidate compounds from squalene and hexadecanoic acid were 8.36% and 5.37%, respectively.

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