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Isolation and Elucidation Structure of the Chemical Component from Ethyl Acetate Subfraction of Kelakai Stems (*Stenochlaena palustris*)

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Abstract

Kelakai (*Stenochlaena palustris)* belongs to the *Blechnaceae* family and offers numerous health benefits due to its chemical components. The isolation and structure elucidation of the chemical components from the ethyl acetate subfraction of the kelakai stems (*Stenochlaena palustris*) have been carried out. The isolation process began with the preadsorption of 241 mg of the E5.6 subfraction, which was then subjected to separation and purification using column chromatography. Three solvent combinations were used in the process: (chloroform 100%, chloroform: methanol (9:1), and chloroform: methanol (8:2)). The choice of solvent was based on the stain patterns observed in thin-layer chromatography (TLC). The chemical component was successfully isolated after chromatography, resulting in a white needle crystal weighing 15.2 mg and a single spot on the TLC, which was monitored using ultraviolet light at a wavelength of 254 nm. The structure of the chemical component was elucidated using ^{1}H-NMR and ^{13}C-NMR spectra, and comparison with literature data confirmed that the isolated compound from the ethyl acetate subfraction of the kelakai stems is succinic acid.

Keywords: *Ethyl acetate subfraction, isolation, Stenochlaena palustris, kelakai stems, succinic acid.*

Introduction

All parts of a plant, including roots, stems, twigs, leaves, flowers, and fruits, contain chemical components. Chemical components are classified into two main groups: primary metabolites and secondary metabolites. Primary metabolites include carbohydrates, proteins, fats, vitamins, and enzymes. Secondary metabolites encompass a diverse range of compounds, including steroids, terpenoids, alkaloids, coumarins, phenolics, and flavonoids. The exploration of the chemical components of plants is essential as it aims to determine the bioactivity of secondary metabolite compounds. These compounds have been widely reported to be useful for humans, serving as natural alternatives in cancer treatment, hepatoprotective agents, antioxidants, antibacterial agents, and anti-inflammatory agents. Additionally, in the field of agriculture, these compounds can be utilized as insecticides, fungicides, and herbicides that are based on natural, environmentally friendly principles[1]–[4].

The plant known as kelakai is a familiar sight in the city of Banjarmasin, South Kalimantan. It is consumed as a vegetable or snack on a daily basis by the local population, who believe that

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it offers health benefits. Consequently, all parts of kelakai have been researched for their natural product chemistry. One of them is the leaves either their young or old leaves. Both leaves have been empirically proven to offer health benefits, particularly in preventing Alzheimer's disease through the inhibition of cholinesterase, α-glucosidase bioactivity to regulate hyperglycemia, and the exertion of antibacterial (antimicrobial) and antioxidant effects[2]–[7].

Our research findings have been published, focusing on the ethyl acetate fraction. Following a series of separation processes and the purification of chemical components from the ethyl acetate fraction of the kelakai stems, a compound known as a molting hormone (20-hydroxyecdysone) for crustacean animals was obtained. The compound was then modified to yield a 20-hydroxyecdysone derivative compound, which was subjected to a structure-activity relationship (SAR) analysis to assess its activity against *Coptotermes curvignathus* termites[8],[9]. However, this research was limited to the 20-hydroxyecdysone compound obtained from one subfraction of the ethyl acetate fraction. Further research could explore the subfractions of other chemical components in the ethyl acetate fraction, which would enrich the data and knowledge of the chemical components contained in the kelakai stems (*S. palustris*).

The potential for obtaining chemical components based on the stain profile of the ethyl acetate subfraction in thin-layer chromatography (TLC) has yet to be fully explored. Therefore, this study aims to isolate chemical components from the ethyl acetate subfraction of kelakai stems.

Experimental

Equipment

The equipment utilized is glassware that is commonly employed in the field of natural product chemistry research, including glass funnels, stirring rods, dropper pipettes, column chromatography with a diameter of 10 mm and a length of 30 cm, desiccators, and a JEOL ECA 400 NMR spectrophotometer.

Material

The chemicals utilized in this study are chloroform and methanol solvents procured from WAKO. The solvents employed for TLC and column chromatography are 100% chloroform, a chloroform: methanol (9: 1) solution, and a chloroform: methanol (8: 2) solution, silica Gel 60 N (spherical, neutral, 40–50 µm, KANTO Chemical Co., Inc.), TLC plate (silica gel 60 F_{254}, Merck KGaA Darmstadt, Germany) with ultraviolet lamp 254 nm and deuterium-methanol solvent (CD_3OD). The ethyl acetate subfraction (E5.6) of *S. palustris* stems was utilized in this study. This subfraction has never been employed in previous studies[8],[9].

Methods

Isolation

The ethyl acetate subfraction (E5.6) obtained from previous studies[8],[9] was monitored for a separation pattern by thin layer chromatography (TLC) with various eluent combinations to visualize the purification processes that could be achieved through column chromatography. The ethyl acetate subfraction (E5.6) weighing 241 mg was pre-absorbed with silica gel before being used for separation by column chromatography. The separation and purification were carried out using column chromatography with silica gel as the stationary phase and a combination of three solvents in the mobile phase: 100% chloroform, chloroform: methanol (9:1), and chloroform: methanol (8:2). The column results were collected in vials, each of which was monitored by TLC and any similar patterns were collected and combined in the same vial. In one of the vials, a single spot was observed on TLC, indicating a pure compound. After evaporation and drying, white needle crystals were found in the vial, which then was stored overnight in a desiccator and analyzed with proton and carbon nuclear magnetic resonance (NMR) spectroscopy.

Results and Discussion

This research is a follow-up study[8],[9] that aims to explore, reveal, and identify the chemical components present in the ethyl acetate fraction of kelakai stems (*S. palustris*). The ethyl acetate subfraction was pre-absorbed to a maximum of 241 mg in order to ensure a firm bond between the sample and the stationary phase[10]. This procedure represents the initial stage of column chromatography (CC). The CC process was conducted with the stationary phase being silica gel and the mobile phase comprising three eluent combinations: 100% chloroform, followed by chloroform: methanol (9:1), and chloroform: methanol (8:2). The selection of the mobile phase was based on the separation pattern observed when monitored by TLC. The chromatography column successfully isolated a pure compound in the form of white needle crystals (15.2 mg), which had been dried using a desiccator to remove the solvent residue.

The chemical structure of pure compounds or chemical components that have been isolated is then elucidated. This is achieved through the use of nuclear magnetic resonance (NMR) spectroscopy, specifically proton (^{1}H-NMR) and carbon (^{13}C-NMR) spectroscopy, using a deuterium-methanol solvent (CD_3OD). The NMR spectra display the chemical shifts of the protons and carbons in the isolated compounds (Figure 1), indicating the presence of several protons and carbons in the compound isolation results[11],[12].

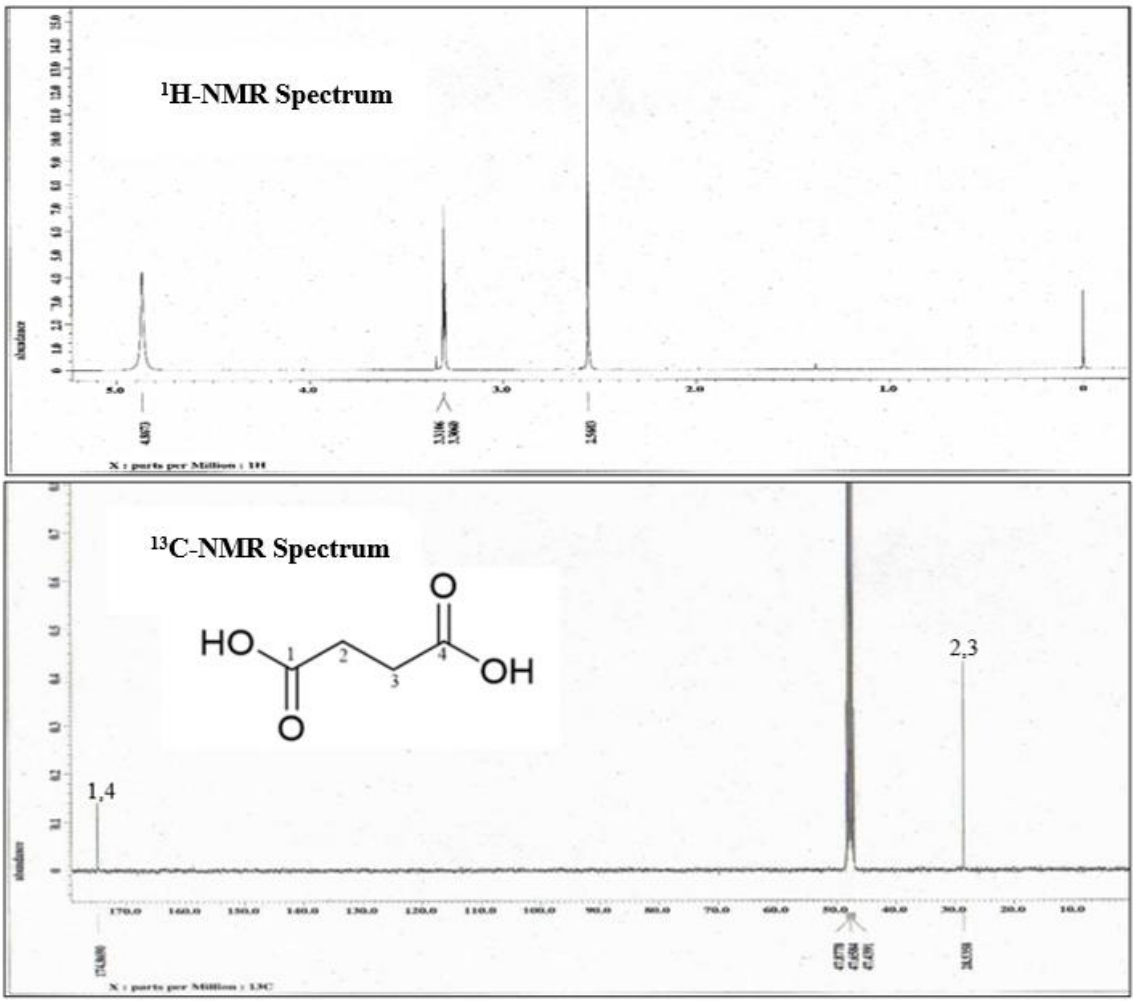


Figure 1. ^{1}H-NMR and ^{13}C-NMR spectra of the compound isolated from the stems of *S. palustris*, using deuterium-methanol (CD_3OD) as the solvent.

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Table 1. Chemical shift data of ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz)

No.	Isolated Compound (CD_3OD)		Succinic Acid (CD_3OD)[13]–[16]	
	δ_C	δ_H	δ_C	δ_H
1	174.86		173.6	
2	28.54	2.56, s	28.7	2.41, s
3	28.54	2.56, s	28.7	2.41, s
4	174.86		173.6	

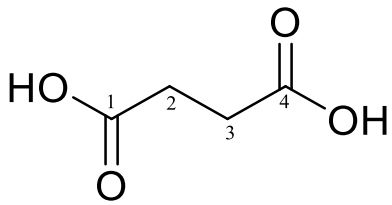


Figure 2. Structure of the isolated compound (succinic acid)

The ^{1}H-NMR spectrum, obtained using a 400 MHz spectrometer (Figure 1), reveals the presence of a chemical shift at δ_H 2.56 ppm (singlet, 4H, H-2 and H-3), indicating the correlation of four hydrogen atoms and two methylene groups (CH_2) as it was confirmed through data from the literature (Table 1). Moreover, a proton signal at δ_H 3.3 ppm was confirmedly coming from the solvent used, deuterium-methanol (CD_3OD). A proton signal is observed at a chemical shift of δ_H 4.87 ppm (singlet, 2H), which is closely associated to the hydroxy group (-OH) in the isolated compound structure. The positions of the hydroxy groups are determined to be C-1 and C-4, based on the proton NMR of succinic acid compounds that have been previously reported[13]–[16].

The ^{13}C-NMR spectrum, obtained at 100 MHz (Figure 1), reveals that the pure compound successfully isolated has a simple structure. This is evident from the spectrum, which exhibits only three signals, two of which are attributed to the isolated compound and one to the solvent used. The chemical shift at δ_C 28.54 ppm indicates the presence of two methylene carbon atoms (CH_2) at positions C-2 and C-3 derived from a comparison of scientific data reported in Table 1. Moreover, a signal at a chemical shift of δ_C 47.66 ppm can be attributed to a carbon atom from the solvent employed, specifically deuterium-methanol (CD_3OD). The isolated compound contains a carbonyl carbon (C=O), as evidenced by the presence of a distinctive and specific signal in the ^{13}C-NMR spectrum at δ_C 174.86 ppm for carbon C-1 and C-4. The isolated compound from the ethyl acetate subfraction of the kelakai stems (*S. palustris*) has a simple and symmetrical structure (Figure 2). The presence of simple and symmetrical structures in compounds results in carbon atom signals that exhibit identical chemical environment conditions, which consequently manifest as a single chemical shift in the carbon NMR spectrum. This phenomenon has been observed in numerous studies, as referenced in the literature[17]–[20]. The carbon atoms C-1 and C-4 have the same chemical environment, resulting in a single signal in the carbon NMR spectrum at δ_C 174.86. Similarly, the carbon atoms C-2 and C-3, which also have the similar chemical environment, produce a carbon signal which appeared only at a chemical shift of δ_C 28.54 ppm.

The ^{1}H-NMR and ^{13}C-NMR spectrum data obtained, along with the comparison of chemical shifts between isolated compounds and succinic acid from published research

reports, yielded the same data correlation, which supports and strengthens the conclusion that the isolated compound from the ethyl acetate subfraction (E5.6) from the kelakai stems (*S. palustris*) is succinic acid.

Conclusions

The chemical component of the ethyl acetate subfraction of kelakai stems (*S. palustris*) has been successfully isolated. Structure elucidation using proton (^{1}H-NMR) and carbon (^{13}C-NMR) spectroscopy along with a comparison of the scientific literature data, has led to the identification and confirmation of the chemical component as succinic acid.

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Declaration of Generative AI and AI-Assisted Technologies

The authors declare that no generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Author Contributions

A.P.: Conceptualization, methodology, investigation, data collection, formal analysis, data curation, visualization, writing —original draft preparation, and corresponding author responsibilities. R.R.A.A.K.W.: Methodology, validation, supervision, and writing —review and editing. Both authors have read and approved the final version of the manuscript.

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Declaration of Conflicting Interests

The authors declare no conflict of interest.

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