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Structural Elucidation of Alkaloid Indole-3-Aldehyde from the Leaves of Kepinding Plant (*Litsea elliptica* Blume)

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ABSTRACT. *Litsea elliptica* Blume has been found in its natural habitat to grow up to 44 m in height and 78 cm in diameter. Traditionally, this plant has been utilized as a vegetable, a food seasoning, in traditional medicine, and as a bed bug repellent. Phytochemical studies have been conducted on this plant's leaves; however, structural investigations of leaf compounds remain limited. The methanolic extract of *Litsea elliptica* Blume leaves was subjected to liquid–liquid partitioning using dichloromethane and subsequently fractionated using various chromatographic techniques, including C18-HPLC, to isolate pure compounds. This study aimed to isolate and purify compounds from the dichloromethane fraction of *Litsea elliptica* Blume leaves and to identify their secondary metabolites. The chemical structures of the isolated compounds were determined based on analyses of 1D and 2D NMR spectroscopic data (correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC), and nuclear Overhauser effect spectroscopy (NOESY)) and mass spectroscopy (MS). Comparison with literature data revealed that the leaf isolate of kepinding is an alkaloid compound with an indole-3-aldehyde structure, which is reported for the first time from *Litsea elliptica* Blume.

INTRODUCTION

Kepinding plant is native to Southeast Asia, including Indonesia, and is commonly used as a vegetable, flavoring ingredient, and traditional herbal medicine (Phoopha *et al.*, 2020; Kuspradini *et al.*, 2024). Its usefulness is evidenced by research on kepinding leaves, which are safe to use as herbal medicines, vegetables, and flavoring agents, based on toxic effects assessed in red blood cells of Sprague-Dawley rats (Taib *et al.*, 2009). Plants of the tribe *Lauraceae* are widely found in the regions of East Kalimantan, Riau, the states of Malaysia (Selangor and Sarawak), Trang Province (Thailand), and Brunei Darussalam (Grosvenor *et al.*, 1995; Budin *et al.*, 2012; Zhang *et al.*, 2014; Goh *et al.*, 2017; Mala *et al.*, 2023). Kepinding plant is also known by various local names. In southern Thailand, it is called tham mang and is used as a vegetable, a flavoring, and in traditional medicine (Phoopha *et al.*, 2020). In Brunei Darussalam, it is known by its local names Stuttgart, Madang Perawas, or tham-mung, and is used by the local community as a medicine to prevent headaches, ulcers, fevers, and cancer (Goh *et al.*, 2022). The Malaysian community knows it by its nickname, São Paulo or Stuttgart, which is used as medicine, in perfume, and for essential oils (Azhar and Salleh, 2020). In Riau Province, it is known as *Madang Perawas,* which is used as a headache remedy (Grosvenor *et al.*, 1995).

Research on Kepinding plant leaves has been widely reported. Pharmacological studies state that *L. elliptica* Blume showed cytotoxicity to cancer cells carcinoma A549, anti-alpha-glucosidase, anthelmintic, antioxidant, and antimicrobial activity (Budin *et al.*, 2012; Zhang *et al.*, 2014; Phoopha *et al.*, 2020; Goh *et al.*, 2022; Kuspradini *et al.*, 2024; Shahlehi and Kasah, 2024). Stubble plants (*L. elliptica* Blume) in the genus Litsea can also be found in Nanga Tayap District, Ketapang Regency, where their leaves and twigs are traditionally used by the local

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community to repel bedbugs, namely kepinding (*Cimex lectularius* L.). These uses are in line with research by Jantan *et al*. (1996), who found that the leaves of *L. elliptica* serve as a repellent against mosquito larvae of *Anopheles maculatus, Aedes aegypti,* and *Culex quinquefasciatus.*

The main content of secondary metabolites in the results of leaf phytochemical screening of *L. elliptica* Blume contains alkaloids, flavonoids, tannins, steroids, and saponins (Larasati *et al.*, 2024). Phoopha *et al.* (2020) reported the successful isolation of secondary metabolites from Thai flowering plants, including alkaloids, flavonoid glycosides, and steroids. Successfully identified compounds include quercetin-3-O-α-ramnopiranoside-(1→2)-α-apiofuranoside, quercetin-3-O-α-ramnopiranoside (quersitrine), quercetin-3-O-β-glucokopiranoside (isoquersitrin), uridine, isoboldine, reticulin, β-sitosterol-D-glucokopiranoside.

To date, no scientific reports have been found on compounds from *L. elliptica* Blume in West Kalimantan. Meanwhile, research conducted by Phoopha *et al.* (2020) on the isolation of alkaloids from the same species provides an important foundation for the presence of alkaloids in this species. Alkaloids are secondary metabolites with a wide diversity of structures and biological activities, including antimicrobial, anti-inflammatory, antimalarial, and cytotoxic activities, making them promising candidates for development as bioactive compounds and medicinal substances. In addition, leaves, as metabolically active plant organs, often become sites of alkaloid accumulation, especially indole alkaloids, which play a role in the plant's defense mechanisms against pathogens and herbivores. Therefore, based on the description above, it is necessary to isolate and determine the structure of compounds in the alkaloid group of *L. elliptica* Blume from West Kalimantan. This research was carried out in several stages, starting with sample preparation and extraction, followed by separation using fractionation methods, column chromatography, and High-Performance Liquid Chromatography (HPLC). The structure of the pure sample was determined using ^{1}H-NMR, ^{13}C-NMR, Heteronuclear Single Quantum Coherence (HSQC), Correlation Spectroscopy (COSY), Heteronuclear Multiple Bond Correlation (HMBC), and Mass Spectrometry (MS).

RESEARCH METHODS

This study used a series of glass tools, bulbs, a set of vacuum liquid chromatography (VLC) equipment, *chambers*, gravity column chromatography equipment (KKG), an analytical balance (Ohaus), a rotary evaporator (IKA), an HPLC-PDA (Jasco), and NMR spectroscopy (Bruker Avance III HD600). The materials used are various types of organic solvents, including methanol (Merck), dichloromethane (Merck), n-hexane (Merck), thin-layer chromatography (TLC) plate (Merck), silica gel 60 F254 (Merck), silica 60 – 70 *mesh* (Merck), and silica gel 230 – 400 mesh *(Merck).* All materials are stored in tightly closed containers to prevent contamination and damage during the research process.

Litsea elliptica *Blume* Leaf Extraction

Samples of kepinding leaves (*L. elliptica* Blume) were obtained from Nanga Tatapi District, Ketapang Regency, West Kalimantan Province. The determination of the plant was carried out at the Directorate of Scientific Collection Management, National Research and Innovation Agency (BRIN), Cibinong, with determination number B-339/II.6.2/IR.01.02/1/2025. Based on the results of the determination, the sample was identified as a species of *L. elliptica,* which belongs to the *Lauraceae* family.

A total of 3.7 kg of leaf powder was macerated using methanol solvent for 3 × 24 hours at room temperature. After the maceration process was complete, the extract was filtered to separate the filtrate and residue. The resulting filtrate was collected, and the solvent was evaporated on a rotary evaporator until a thick methanol extract formed. Furthermore, the methanol extract was partitioned in a separatory funnel using *n*-hexane. The resulting methanol fraction was then repartitioned using dichloromethane (DCM), yielding an extract weighing 6.2341 g.

Column Chromatography and HPLC

Dichloromethane extract was separated using vacuum liquid chromatography (VLC) technique with a column thickness of 0.25 mm; the diameter size of 4 cm and the elution distance of 10 cm used a combination of solvents by gradient of 100% nhexane: EtOAc (9:1), (8:2), (7:3), (6:4), (1:1), (4:6); (3:7), (2:8), (1:9), 100% EtOAc, and 100% MeOH and produced 16 fractions. Furthermore, a K6 fraction of 482.7 mg with a good separation pattern and a large mass was selected for purification using gravity columns.

Gravity column chromatography was performed using a column with a diameter of 1 cm and a silica height of 12 cm. The K6 fraction (482.7 mg) was eluted with 100% DCM eluents, DCM: MeOH (99:1); (98:2); (97:3);

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(96:4); (95:5); (9:1); and 100% MeOH, so that a fraction (KD1-KD40) was obtained. The K6-7 fraction (4.8 mg) with a good chromatogram separation pattern was selected for purification using HPLC. The HPLC chromatogram used a C18 column (nonpolar stationary phase), with a solvent flow rate (flow rate) of 5 mL/min and a methanol-water solvent gradient from 75% to 100% for 20 minutes.

Compound analysis

K6-7 isolate was analyzed using an infrared spectrophotometer to identify functional groups present in the isolate. The structure of the K6-7 isolate from the chimney plant (*Litsea elliptica* Blume) was analyzed using a spectrophotometer for ^{1}H-NMR (600 MHz), ^{13}C-NMR, DEPT, HSQC, HMBC, and NOESY using CD3OD solvent at Meijo University.

RESULTS AND DISCUSSION

The results of the infrared (IR) spectrum analysis of the K6-7 isolate showed a weak absorption band at 3318 cm^{-1}, indicating the presence of an N–H longitudinal vibration typical of a secondary amine group. A sharp absorption with strong intensity at 2920 cm^{-1} indicates an aliphatic C–H longitudinal vibration, commonly found in methyl or methylene groups. Absorption at 1697 cm^{-1} indicates the presence of carbonyl groups (C=O), which is characteristic of aldehyde groups (–CHO). Absorption in the 1503 cm^{-1} region indicates aromatic C=C longitudinal vibrations in the indole ring system. In addition, absorption at the 1243 cm^{-1} wavenumber indicates C–N stretch vibration, which is one of the characteristics of alkaloid group compounds (Hameed *et al.*, 2000; Akbar *et al.*, 2023).

Phytochemical tests were performed on the dichloromethane (DCM) fraction to detect the presence of secondary metabolites, including flavonoids, alkaloids, steroids, phenolic compounds, saponins, and terpenoids. Test results in Figure 1 showed that the DCM extract of *L. elliptica* gave positive test for alkaloids using the Dragendorff reagent, characterized by the formation of deposits (Ghosal and Mandal, 2012).

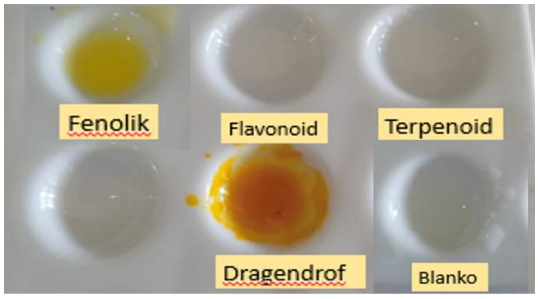


Figure 1. Results of phytochemical test of K6-7 isolate of the dichloromethane fraction of the leaves of the clypine plant (*L. elliptica* Blume).

K6-7 isolates were further analyzed using 1H and 13C NMR spectroscopy, and the results of the interpretation are shown in Table 1.

Table 1. Data of ^{1}H-NMR, ^{13}C-NMR, HSQC, HMBC, and COSY for isolate K6-7.

Position	Proton Chemical Shift (ppm)	δ_C ppm	COSY	HMBC
1				
2	8.08 (*s*,1H)	137.6		C7, C9, C4
3		122.9		
4		136.9		
5	8.13 (*t,t,* 1H, *J=2.2; 2.1)*	120.3	H6	C3, C4
6	7.24, (*d,t,d*, *J=1.2; 1.86; 1.14)*	121.6	H5	C7, C8
7	7.27 (*d,t,d* 1H, *J= 1.32; 2.34; (1.44)*	123.7	H8	C4, C5
8	7.44 (*t,t,* 1H, *J=1.92; 2.04)*	111.1	H7	C6, C7
9		118.1		
10	9.85 (*s*, 1H)	184		C3

Table 1 indicates the presence of characteristic signals of heteroatomic aromatic structures at the chemical shift range δ 6 – 9 ppm (Syah, 2016). Four proton signals were observed in δ_H 7.24 (dtd, J = 1.2; 1,86; 1.14 Hz,

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H-6); δ_H 7.27 (dtd, J = 1.32; 2,34; 1.44 Hz, H-7), δ_H 7.44 (tt, J = 1.92; 2.04 Hz, H-8), and δ_H 8.13 (tt, J = 2.2; 2.1 Hz, H-5). The separation pattern consists of two doublets and two triplets in the range of δ_H 7.24–8.13 ppm, indicating a 1.2 (ortho-substituted) benzene ring system (Kim *et al.*, 1997; Xu *et al.*, 2010). The coupling constant (J) is relatively small, i.e., about 1.2–2.2 Hz, indicating a typical long-range coupling interaction for protons at the meta position in the aromatic system. This reinforces that the substituent in the benzene ring is in proximity (ortho), leaving only the observed meta coupling pattern in the proton.

The signal at δ_H 9.85 (s, 1H, H-10) indicates the presence of aldehyde protons (–CHO) that are strongly deshielded due to the influence of carbonyl groups. This signal is consistent with the characteristics of aldehyde protons that generally appear at δ_H 9–10 ppm. Meanwhile, the presence of a singlet signal at δ_H 8.08 (1H, H-2) indicates an olefinic proton metin located in a heterocyclic ring. The absence of separation (singlet) in this proton indicates that it does not have a neighboring proton that can be coupled, which is consistent with the structure of the substituted heteroaromatic system. Overall, these ^{1}H-NMR spectrum data support the existence of an ortho-substituted aromatic ring framework conjugated to aldehyde groups and heterocyclic systems.

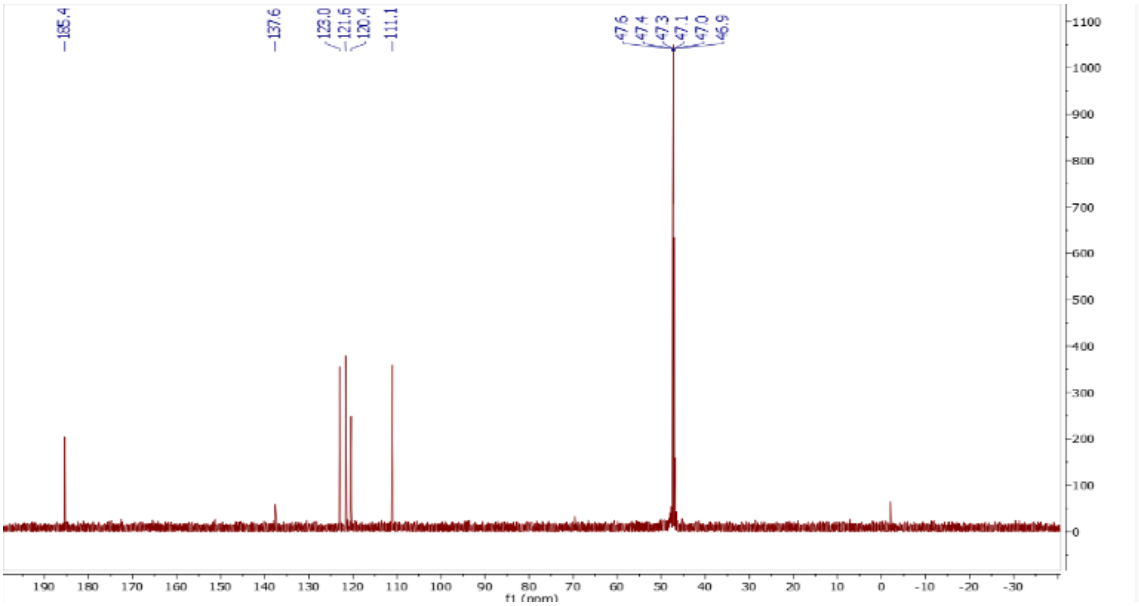


Figure 2. DEPT spectrum 135.

The ^{13}C NMR spectrum and DEPT-135 show the presence of nine resonances for one carbonyl group and eight olefinic carbons (Xu *et al.*, 2010). Six signals for carbon methine (δ 184.5 (C10); 121.6 (C6); 120.38 (C5); 123.73 (C7); 111.11 (C8); 137.62 (C2)) and three quaternary carbons are present in δ 118,15 (C9); 136,94 (C4); and 122.99 (C3) (Syah, 2016). The ^{13}C NMR spectrum and DEPT-135 support the structural character of indole-3-aldehyde, with the aldehyde carbon signal at δ 187.42 ppm indicating that the C10 carbon is deshielded. Shifts in aromatic carbons at 120-140 ppm indicate carbons in the benzene ring. Carbon positions closer to heterocyclic nitrogen atoms tend to experience stronger deshielding (seen at δ 130 – 139 ppm) (Rout *et al.*, 2021).

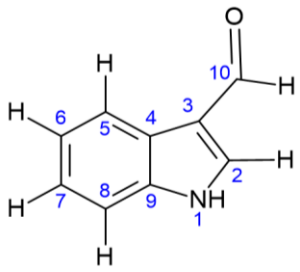


Figure 3. Structure of indole-3-aldehyde.

The approach to determining the structure involved comparing the results of previous studies that successfully isolated indole-3-aldehyde (Figure 3). Yue *et al.* (2000) isolated the indole-3-aldehyde compound from the endophytic fungus *Epichloe festucae*, Inoue *et al.* (2004) from Diaion resin HP-20, Xu *et al.* (2010) from the

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mushroom *Volvariella bombycina*, and Liao *et al*. (2014) from the fruit of the *Capparis masaikai.* The differences in reported chemical shifts are likely influenced by variations in isolation conditions and the type of solvent used, as both can alter intermolecular interactions and the structural stability of compounds (Hasan, 2021).

The indole-3-aldehyde structure is supported by COSY and HMBC data. COSY provides information on the connectivity of protons in close proximity, while HMBC reveals the long-range correlation between protons and neighboring carbons separated by two to three bonds. Consequently, HMBC is highly useful for determining and confirming the positions of protons relative to their neighboring carbons in the carbon skeleton.

The HMBC data for methine protons at δ 7.24 (H6) correlated with olefinic carbons at δ 123.73 (C7) and δ 111.1 (C8). The methine protons at δ 7.27 (H7) correlated with olefin carbons at δ 120.38 (C5) and δ 136.94 (C4). Proton metin δ 7.44 (H8) correlated with δ 121.6(C6), and δ 123.73(C7). Proton metin δ 8.08 (C2) correlated with δ 123.73 (C7), δ 118.15 (C9), and δ 136.94 (C4). The methine protons at δ 8.13 (H5) correlate with δ 122.99 (C2) and δ 136.94 (C4). The methine proton of an aldehyde at δ 9.85 (H10) correlates with the quaternary carbon δ 122.99 (C3). In COSY, the methine protons in the olefinic carbon δ 7.44 (H8) are neighbored by δ 7.27 (H7), while the methine protons at δ 7.24 (H6) are neighbored by δ 8.13 (H5); both make up the benzene ring section. The correlations in HMBC and COSY are shown in Figures 4 – 5 and Table 2.

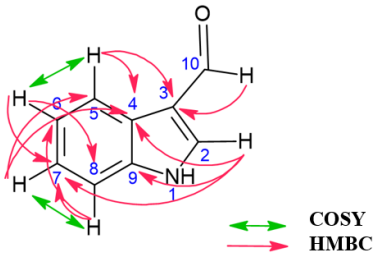


Figure 4. Correlation of ^{1}H-^{1}H COSY and HMBC for indole-3-aldehyde compounds (^{1}H-indole-3-carbaldehyde).

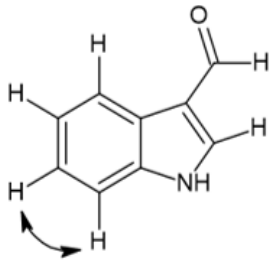


Figure 5. Correlation of NOESY Indole-3-aldehyde Compounds.

Table 2. Comparison of chemical shift (δ) between isolate and indole-3-aldehyde.

	K6-7		Resin Diaion HP-20	
	600 MHz CD3OD	**δC ppm**	**500 MHz, MeOHd6**	**δ C13 ppm**
1				
2	8.08 (*s*,1H)	137.62	8.10 (1H, *s*)	139.9
3		122.99		125.9
4		136.94		139.1
5	8.13 (*t,t*, 1H, *J=2.2; 2.1)*	120.38	8.16 (1H, *d*, *J=8.0 Hz,)*	113.3
6	7.24 (*d,t,d*, *J=1.2; 1.86; 1.14)*	121.60	7.48 (1H, *d*, *J=8.0 Hz,)*	122.6
7	7.27 (*d,t,d* 1H, *J= 1.32; 2.34; 1.44)*	123.73	7.28 (1H, *d,t*, *J=1.0, 8.0 Hz,)*	125.2
8	7.44 (*t,t*, 1H, *J=1.92; 2.04)*	111.11	7.42 (1H, *d,t*, *J=1.0, 8.0 Hz)*	123.8
9		118.15		120.3
10	9.85 (1H,*s*)	184.5	9.86 (1H,*s*)	187.5

The NOESY (Nuclear Overhauser Effect Spectroscopy) spectrum (Figure 5) provides information about the spatial interactions between adjacent protons (<5 Å). The indole-3-carboxaldehyde structure exhibits a cross-peak between H-7 and H-8. The emergence of this signal demonstrates that these two protons are in close spatial proximity (a through-space interaction), even though they are not directly connected through bonds. This spatial interaction reflects the characteristic orientation of adjacent protons on a benzene ring.

Although indole-3-carboxaldehyde has been isolated from various sources, this is the first report of its occurrence in the species *L. elliptica* Blume, as well as within the literature of the *Lauraceae* family. Liao *et al.* (2014) have isolated the indole-3-aldehyde compound from the fruit part of the species *Capparis masakai* (family *Capparaceae*). Indole-3-aldehyde was also successfully isolated from endophytic fungi, HP-20 Diaion resin, sponges, and fungi (Yue *et al.*, 2000; Inoue *et al.*, 2004; Samchai *et al.*, 2011; Shady *et al.*, 2017; Zhou *et al.*, 2022). The structure of the isolated compound was confirmed by mass spectrometry, demonstrating similarity to

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previous studies. As reported by Inoue *et al*. (2004), indole-3-aldehyde shows a peak at *m/z* 145 $[M+H]^+$, while Yue *et al.* (2000) reported a peak at *m/z* 145 $[M]^+$. The compounds produced by the K6-7 isolate showed a peak at *m/z* 146.0599 $[M+H]^+$. These results confirmed that the K6-7 isolate compound had the same mass as indole-3-aldehyde. The biological activity of the compound indole-3-aldehyde has been reported in various previous studies. Isolation of indole-3-aldehyde from the bacterium *Pseudomonas fluorescens,* as reported by Zhou *et al.* (2022) shows repelling activity against *Caenorhabditis elegans.* Inoue *et al.* (2004) report that indole-3-aldehyde may inhibit bacterial growth of *Legionella pneumophila.*

CONCLUSION

Indole-3-aldehyde in the form of yellow crystalline solids has been successfully isolated. The supporting data are NMR-^{1}H (600 MHz, CD_3OD), DART-MS *m/z*: 146.0599 $[M+H]^+(C_9H_7ON)$. Information on the discovery of indole-3-aldehyde alkaloid compound in the species *L. elliptica* Blume was first reported in this study, providing important insights for the study of *L. elliptica* Blume from a taxonomic perspective.

CONFLICT OF INTEREST

There are no conflicts of interest in this article.

AUTHOR CONTRIBUTION

YP: Manuscript Draft Writing, Data Collection, Data Analysis; AHA: Conceptualization, Methodology, Supervision, Manuscript Review and Editing; R: Conceptualization, Methodology, Supervision, Review and Manuscript Editing; YT: Conceptualization, Supervision, Review.

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DECLARATION OF GENERATION AI

No declaration of generative AI use was provided.

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