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Phytochemical investigation of *Calophyllum sundaicum* P.F.Stevens: isolation, structure elucidation, and biological potential

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ABSTRACT

Despite the abundance of xanthenes in the *Calophyllum* genus, the stem bark of *C. sundaicum* remains understudied. Hence, this study focused on the extraction, isolation, and characterisation of secondary metabolites from *C. sundaicum* stem bark and the determination of their biological properties. A newly isolated xanthone, sundaixanthone A (**1**), along with six known xanthenes (**2-7**), were structurally elucidated based on 1D/2D NMR, FTIR, and MS analyses. The methanolic extract exhibited the highest TPC (283.8 ± 8.65 mg GAE/g) and strongest antioxidant activity (IC_{50} 6.39 ± 0.47 μ g/mL), while the chloroform extract demonstrated the highest TFC (116.0 ± 0.66 mg QE/g). Moreover, ethyl acetate and methanolic extracts displayed the lowest MIC values (0.056 mg/mL) against Ab10. Molecular docking revealed a strong binding of sundaixanthone A to PBP (-7.5 kJ/mol), exceeding imipenem (-6.0 kJ/mol). These results highlight that *C. sundaicum* is a promising source of antioxidant and antibacterial agents.

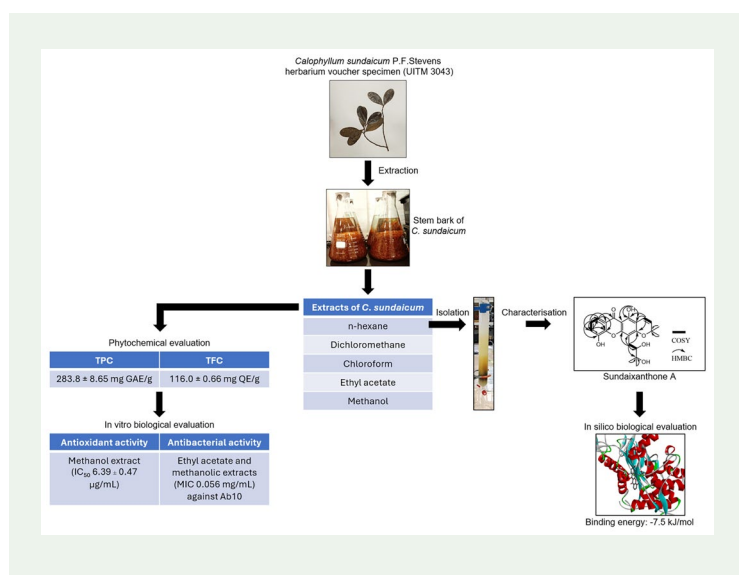
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1. Introduction

Calophyllum sundaicum P.F.Stevens tree is a member of *Calophyllum* genus, one of the largest genera within the family Calophyllaceae. This evergreen tree is distributed in Southern Peninsula Malaysia, Singapore, Borneo (Sarawak, Brunei, West Kalimantan), and Sumatera Island (Stevens 1980; Gunawan et al. 2012; Randi et al. 2014; Zamri and Slik 2018; Seah et al. 2019), thriving in low ground peat swamps up to 40 metres above sea level. The epithet 'sundaicum' originates from the area it occurs frequently which was previously part of Sundaland, and it is locally known as 'bintangor batu' in Malaysian. The fruiting typically occurs in March and May, with flowering between June and September (Stevens 1980). The phytochemical and pharmacological potential of *C. sundaicum* is largely underexplored despite its ecological significance. The genus *Calophyllum* is renowned for its wide array of secondary metabolites, especially from the class of xanthenes and coumarins, which are linked with a variety of biological activities (Ee, 2004). For example, antimicrobial, antiviral, anti-inflammatory, and cytotoxic activities (Chan, 2012; Karunakaran, 2016; Zailan et al. 2022). Up to the present, a limited study has been conducted on *C. sundaicum*. The isolation of sundaicumones A and B from leaves were reported to exhibit weak activity towards the glucocorticoid receptor (Cao et al. 2006). Nevertheless, the studies on the stem bark of the species remain scarce, emphasising the research gap in understanding its chemical composition and bioactivity profile.

The global health crisis posed by multidrug-resistant (MDR) bacteria, especially *Acinetobacter baumannii*, a well-known nosocomial pathogen with few available treatments, highlights the urgency of such research. It develops the ability to resist multiple antibiotics and poses a significant threat in clinical settings (Shi et al. 2024). Historically, natural products have been a good source of novel therapeutic candidates (Chaachouay and Zidane 2024). Thus, exploring the phytochemical profile of *C. sundaicum* may provide valuable information and might potentially reveal compounds with

antibacterial properties. Mechanistically, antibacterial agents such as β -lactam target penicillin-binding protein (PBP), which is a crucial enzyme in the biosynthesis of bacterial cell walls. Inhibition of PBP weakens the integrity of the cell wall and causes bacterial lysis, thus, regarded as a practical approach to treating gram-negative bacteria (Sethuvel et al. 2023). Computational docking is a potent platform for predicting and visualising such molecular interactions, allowing insights into the antibacterial potential of the newly isolated compound.

The current study aims to bridge the research gap through (i) extraction, isolation, and characterisation of compounds from the stem bark of *C. sundaicum*, (ii) evaluation of phytochemical profile, antioxidant capacity and antibacterial potential of the extracts, and (iii) evaluation of the antibacterial mechanism of the new compound through molecular docking. The findings of this research contribute to the understanding of the *Calophyllum* genus and underscore the potential of *C. sundaicum* as a promising source of bioactive compounds for pharmaceutical development.

2. Results and discussion

2.1. Extraction and isolation of *C. sundaicum* stem bark

The sequential maceration extraction of *C. sundaicum* stem bark has yielded 26.7 g of oily yellowish-brown extract (CSuH) from *n*-hexane, 78.5 g of yellowish-brown extract (CSuD) from dichloromethane, 11.6 g of yellowish-dark brown extract (CSuC) from chloroform, 12.4 g of brownish-black extract (CSuEA) from ethyl acetate, and 26.7 g of reddish-black extract (CSuM) from methanol. The percentage yield of the extracts was tabulated in Table 1. The isolation and purification of CSuH have yielded a new xanthone, sundaixanthone A (**1**), along with other known xanthones, 1-hydroxy-7-methoxyxanthone (**2**), trapezifolixanthone (**3**), ananixanthone (**4**), 1,5-dihydroxy-3-methoxy-4-isoprenylxanthone (**5**), 2-hydroxyxanthone (**6**), and a triterpenoid, stigmasterol (**7**), as shown in Figure 1.

2.2. Characterisation of sundaixanthone A (**1**)

Compound **1** was isolated as a pale yellow amorphous with a melting point of 213–214°C. The LC-MS spectrum gave a molecular peak at *m/z* 412.15. The IR spectrum displayed absorptions at 3393 (OH group), <3000 (sp^3 C-H stretch), 1739 (conjugated C=O), and 1650 and 1584 (aromatic C=C) cm^{-1} . The ^1H NMR spectra showed signals for a chelated hydroxyl group (δH 13.19), and a broad singlet belongs to lone OH

Table 1. Extraction yield of *C. sundaicum* extracts expressed as dry weight (g) and percentage yield (%).

Crude extracts	Dry yield (g)	Percentage yield (%)
CSuH	26.7	1.78
CSuD	78.5	5.23
CSuC	11.6	0.77
CSuEA	12.4	0.83
CSuM	26.7	1.78

CSuH: *n*-hexane extract; CSuD: dichloromethane extract; CSuC: chloroform extract; CSuEA: ethyl acetate extract; CSuM: methanol extract.

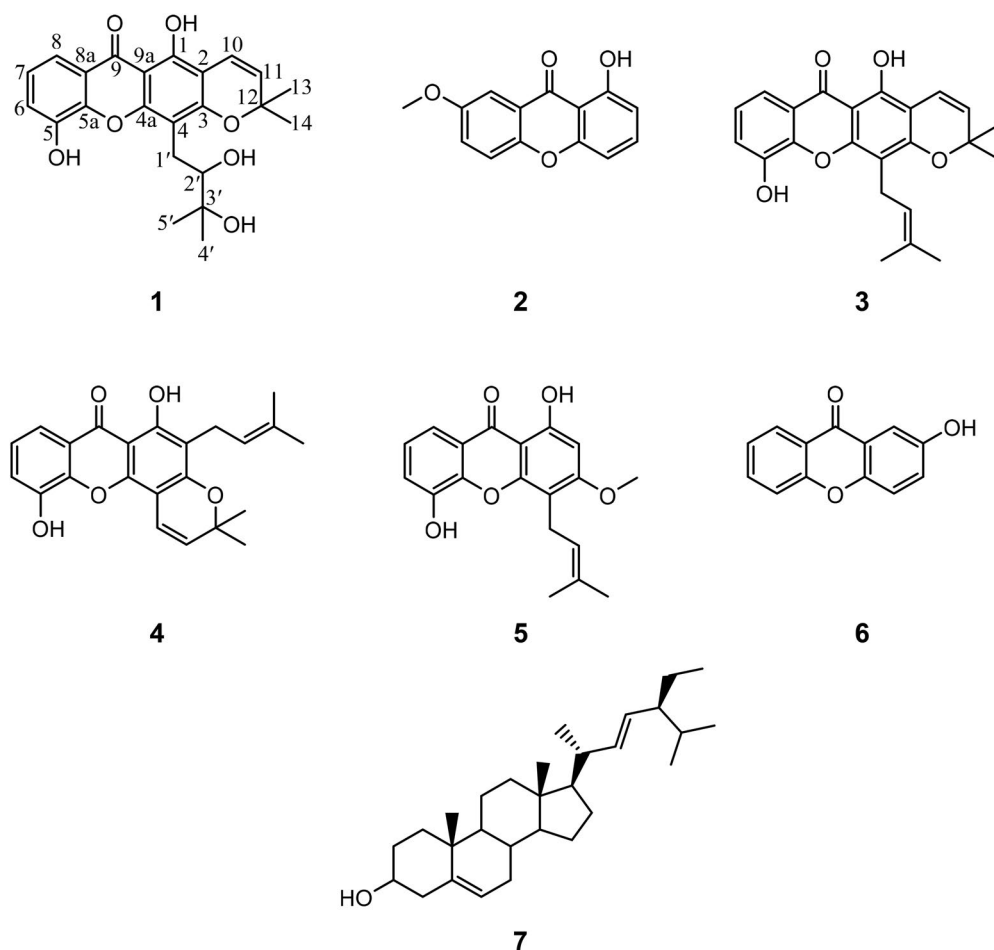


Figure 1. Chemical structures of compounds 1-7.

(δ H 9.10). The two olefinic protons (δ H 5.78 and 6.73) and two singlet protons (δ H 1.51 and 1.52) formed a 2,2-dimethylpyran ring. The coupling of aromatic protons at δ H 7.29, 7.33, and 7.66 with each other was observed in the COSY spectrum, indicating the presence of a benzene ring. A comparison was made (Table S1) between the 1D NMR spectral data of compound 1 and trapezifolixanthone (Lizazman et al. 2023) suggesting that the new compound has a similar skeleton to that of trapezifolixanthone. The remaining protons were assigned to a 2,3-dihydroxy-3-methylbutyl chain and were further proven by the HSQC and HMBC experiments. In addition, the spectral data of the 2,3-dihydroxy-3-methylbutyl chain was compared with the literature (Guilet et al. 2001), confirming the presence of this moiety.

The C=O signal was observed as the most shifted quaternary carbon peak at δ C 181.2 in the ^{13} C NMR spectrum. The DEPT (45, 135) experiments concluded the presence of twelve quaternary carbons, six methine carbons, a methylene carbon and four methyl carbons which is in accordance with molecular formula $C_{23}H_{24}O_7$. Correlations among the protons and their corresponding carbons were established

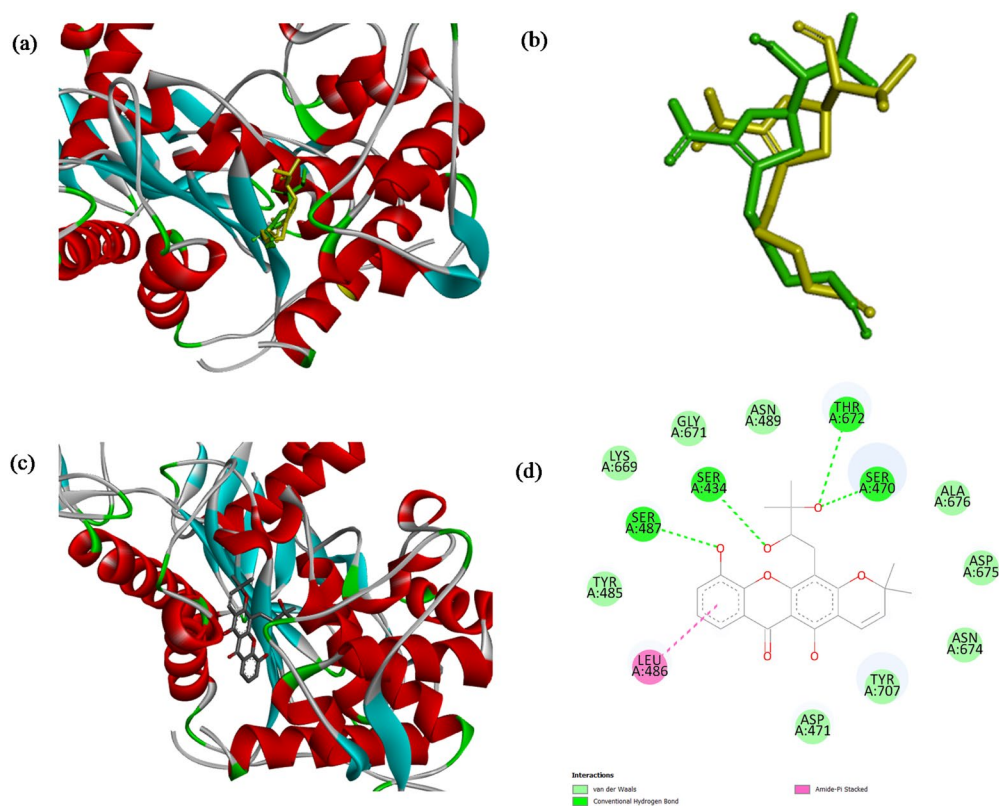


Figure 2. Diagrams showing (a) the alignment of the redocked imipenem (green stick model) and imipenem co-crystallized (yellow stick model) within the binding site of PBP; (b) superimposition of redocking imipenem (green stick model) and imipenem crystal (yellow stick model); (c) sundaixanthone A within the binding site of PBP; (d) the 2D schematic diagram of residues in the binding site of PBP exhibiting hydrogen bond and hydrophobic interaction with sundaixanthone A visualised and analysed by using discovery studio 4.0.

in the COSY and HMBC spectra and illustrated in [Figure 2](#). The protons were assigned to their respective carbons according to HSQC spectrum.

The HMBC spectrum of compound **1** showed the connectivity between the protons and carbons in the range of two to four bonds. The aromatic proton appearing at δ H 7.33 (H-6) was correlated with carbons at δ C 116.1 (C-8), 146.0 (C-5a), and 147.1 (C-5), while the proton appearing at δ H 7.29 (C-7) was correlated with δ C 116.1 (C-8), 121.9 (C-8a), 121.4 (C-6), 146.0 (C-5a), and 147.1 (C-5). Meanwhile, the proton at δ H 7.66 (H-8) gave a correlation with C-6, C-5a, and C-9, thus, establishing the nature of the benzene ring. The cross-peaks of respective H-10 with C-1, C-2, and C-3, and H-11 with C-2 agreed with a linear arrangement of the pyrano unit.

The signal of the chelated OH group at δ H 13.19 with carbons at δ C 104.0, 105.1, and 156.6 suggested its location at C-1. The correlation between proton H-1' with C-3, C-4, and C-4a allowed the position of the 2,3-dihydroxy-3-methylbutyl chain at C-4 of the xanthone moiety. Thus, this compound was established as 12-(2,3-dihydroxy-3-methylbutyl)-5,10-dihydroxy-2,2-dimethyl-2H,6H-pyrano[3,2-*b*] xanthen-6-one,

with a trivial name, *sundaixanthone A*. The FTIR, LCMS, 1D and 2D NMR spectra, and 2D NMR correlations of *sundaixanthone A* are shown in **Fig. S1-S8** in the [supplementary material](#).

2.3. Total phenolic content (TPC) of *C. sundaicum* extracts

TPC value was expressed in milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g extract). The TPC of the *C. sundaicum* extracts ranged from 77.50 ± 3.04 mg GAE/g in CSuH to 283.8 ± 8.65 mg GAE/g in CSuM, with significant differences ($p < 0.05$) across the various solvent fractions. The phenolic concentration in CSuM was the greatest and differed greatly from that of the other extracts. A moderate amount of phenolic compounds was detected in CSuC and CSuD extracts, with respective 99.08 ± 3.48 , and 86.08 ± 3.50 mg GAE/g. However, the ethyl acetate extract (CSuEA) had a significant quantity of phenolics (223.50 ± 4.20 mg GAE/g). Moreover, a non-polar solvent is less effective in extracting phenolics from the sample, as observed in CSuH of which has the lowest phenolic concentration.

2.4. Total flavonoid content (TFC) of *C. sundaicum* extracts

TFC value was expressed in milligrams of quercetin equivalent per gram of dry extract. Significant differences in the TFC results of the extracts were observed ($p < 0.05$) with values ranging from 13.39 ± 0.24 mg QE/g in CSuH to 116.0 ± 0.66 mg QE/g in CSuC. The highest flavonoid concentration was found in extracts CSuC and CSuEA with no significant difference between them. The CSuD and CSuH extracts revealed relatively low flavonoid levels, while the methanolic extract (CSuM) had a moderate flavonoid concentration (44.69 ± 4.12 mg QE/g). These findings imply that solvents with an intermediate polarity would be better at extracting flavonoids than solvents with a high or low polarity.

2.5. Antioxidant assay (2,2-diphenyl-1-picrylhydrazyl) of *C. sundaicum* extracts

The DPPH free radical scavenging test is a quick and effective way to measure free radical scavenging activity. The change of colour from purple to yellow indicates that the absorption of the DPPH radical has decreased. This demonstrates an interaction between the antioxidants in the extracts and free radicals from DPPH. The IC_{50} value for the DPPH free radical scavenging assay is as follows: Inactive at $4250 \mu\text{g/mL}$; mildly active at $4100\text{--}250 \mu\text{g/mL}$; moderately active at $450\text{--}100 \mu\text{g/mL}$; highly active at $10\text{--}50 \mu\text{g/mL}$; and very active at $<10 \mu\text{g/mL}$ (Nuzul et al. 2022). The IC_{50} value in the DPPH assay (Table 2) indicated the antioxidant activity of *C. sundaicum* extracts demonstrated notable differences ($p < 0.05$) across the extracts. The maximum antioxidant activity was portrayed by the CSuM, with an IC_{50} value of $6.39 \pm 0.47 \mu\text{g/mL}$, which was similar to that of the positive control, quercetin (IC_{50} $6.33 \pm 0.34 \mu\text{g/mL}$). While CSuD and CSuC extracts displayed lower radical scavenging activities, with IC_{50} values of 102.3 ± 9.58 , and $144.6 \pm 4.47 \mu\text{g/mL}$, respectively, CSuEA exhibited a strong antioxidant activity (IC_{50} $14.04 \pm 0.14 \mu\text{g/mL}$). On the other hand, the CSuH with an

IC₅₀ value of more than 1000 µg/mL indicate negligible antioxidant activity. According to these findings, extracts with higher levels of flavonoids and phenolic compounds have greater antioxidant capability, spotlighting the role of these bioactive substances in scavenging free radicals.

2.6. Pearson correlation between TPC, TFC, and antioxidant activity (DPPH)

The relationship between the TPC, TFC, and antioxidant activity of the extracts was investigated using the Pearson correlation (Table 3). The correlation analysis unveiled that the TPC and TFC of *C. sundaicum* extracts varied independently, which exhibited no significant linear association ($r=0.005$, $p=0.994$). A negative nonsignificant connection was observed between TPC and DPPH activity ($r=-0.456$, $p=0.440$). However, the correlation between TFC and DPPH activity showed a stronger negative relationship ($r=-0.773$, $p=0.125$), indicating that increased flavonoid concentration may lead to improved antioxidant activity, even though it is not statistically significant. These findings suggest that several variables influence antioxidant activity and different phenolic and flavonoid substances may have a synergistic effect on radical scavenging capability. The relationship between these phytochemical components and antioxidant activity may be better understood with more research and a bigger sample size (Bujang 2024).

2.7. Antibacterial activity of *C. sundaicum* extracts

The antibacterial activity of five extracts from *C. sundaicum* was assessed against gram-negative bacterial strains of *A. baumannii* (Ab06, Ab10, and Ab10CR), which belong to the same species but were sourced differently. The Ab10 strain was induced with

Table 2. TPC, TFC, and antioxidant activity (DPPH) of *C. sundaicum* extracts.

Extracts	TPC (mg GAE/ g extract)	TFC (mg QE/ g extract)	DPPH (IC ₅₀ µg/mL)
CSuH	77.50 ± 3.04 ^a	13.39 ± 0.24 ^a	>1000 ^a
CSuD	86.08 ± 3.50 ^{a, b}	89.14 ± 0.75 ^b	102.3 ± 9.58 ^b
CSuC	99.08 ± 3.48 ^b	116.0 ± 0.66 ^c	144.6 ± 4.47 ^b
CSuEA	223.50 ± 4.20 ^c	111.2 ± 1.50 ^c	14.04 ± 0.14 ^b
CSuM	283.8 ± 8.65 ^d	44.69 ± 4.12 ^d	6.39 ± 0.47 ^b
Positive control			
Quercetin	–	–	6.33 ± 0.34

Values are expressed as mean ± standard deviation (SD) ($n=3$). Different superscript letters (^{a–d}) indicate statistically significant differences between extracts within the same column as determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

Table 3. Correlation coefficients (r) and p -values between TPC, TFC, and antioxidant activity (DPPH) of *C. sundaicum* extracts.

	TPC		TFC		DPPH	
	Coefficient (r)	p -value	Coefficient (r)	p -value	Coefficient (r)	p -value
TPC	1.000	–	0.005	0.994	–0.456	0.440
TFC	0.005	0.994	1.000	–	–0.773	0.125
DPPH	–0.456	0.440	–0.773	0.125	1.000	–

Table 4. MIC And MBC values (mg/mL) for *C. undaicum* extracts against three *A. baumannii* bacterial strains.

Extracts	Bacterial strains					
	Ab06		Ab10CR		Ab10	
	MIC	MBC	MIC	MBC	MIC	MBC
CSuH	0.900	+	0.450	+	0.113	+
CSuD	0.450	+	0.225	+	0.225	+
CSuC	0.450	+	0.113	+	0.113	+
CSuEA	0.450	+	0.113	+	0.056	+
CSuM	0.450	+	0.113	+	0.056	+

A positive sign (+) indicates bacterial growth, and a negative sign (–) indicates no growth observed for MBC determination.

resistance against Colistin (Ab10CR) while some other strains underwent a resistance induction process. The plant extracts include CSuH, CSuD, CSuC, CSuEA, and CSuM extracts. DMSO was used as the negative control and erythromycin was used as the positive control in this study. The MIC and MBC results were tabulated in Table 4, and are available in the [supplementary materials \(Figs. S9-15\)](#).

According to Demgne et al. (2021), MIC values that are lower than 0.100 mg/mL indicate high activity while MIC values between 0.100 to 0.625 mg/mL suggest moderate activity. In this study, CSuEA and CSuM extracts displayed the most significant antibacterial activity, particularly against Ab10 with MIC values of 0.056 mg/mL. For Ab10CR, both extracts CSuEA and CSuM showed moderate antibacterial activity with MIC values of 0.113 mg/mL, while also showing moderate activity towards Ab06 with MIC values of 0.450 mg/mL. Moreover, CSuC extract demonstrated a moderate activity across all strains, with MIC values of 0.450 mg/mL for Ab06 and 0.113 mg/mL for both Ab10CR and Ab10. This is similar to the CSuD extract, which exhibited moderate activity with MIC values of 0.450 mg/mL against Ab06 and 0.225 mg/mL against both Ab10CR and Ab10. The least active extract was portrayed by CSuH with the MIC value of 0.900 mg/mL for Ab06, suggesting a minimal inhibitory effect while showing moderate activity towards Ab10CR and Ab10 with MIC values of 0.450 mg/mL and 0.113 mg/mL, respectively.

The variability in MIC values among the strains could be attributed to their differences in resistance profiles. Ab06, most likely a multidrug-resistant strain, requiring the highest concentration, 0.900 mg/mL for CSuH extract and 0.450 mg/mL for the rest of the extracts to inhibit their growth, which could be due to the environmental exposure and antibiotic adaptation as explained by their adapted resistance mechanism (Serwecińska 2020). All extracts showed bacteriostatic activity towards the strains tested, as bacterial growth was observed based on the MBC findings, indicating that all the extracts only inhibited the bacterial growth; however, they did not kill them.

2.8. Molecular docking of undaixanthone A

The root mean square derivative (RMSD) value between the top-ranked docked imipenem conformation and with crystal pose of imipenem was found to be 1.6691 Å. The molecular docking results show that undaixanthone A shows a good binding energy compared to β -lactam antibiotics, Imipenem, with a binding energy of -7.5 kJ/

mol. The high binding energy of sundaixanthone A can be attributed to the formation of four (4) hydrogen bonds between sundaixanthone A and the amino acid residues of penicillin-binding protein (PBP) such as SER487, SER434, THR672 and SER470 (Figure 2). The hydrogen bonds are contributed by the oxygen element attached to the sundaixanthone A. Furthermore, sundaixanthone A was found to interact with specific amino acid residues, including ASN489, SER434, LYS669, and TYR707, *via* hydrogen bonds and van der Waals interactions which are also involved in the binding mechanism of β -lactam antibiotics such as penicillin G, imipenem and aztreonam (Han et al. 2011). This similarity suggests that sundaixanthone A may exhibit a comparable mode of action to β -lactam antibiotics, potentially influencing its antimicrobial efficacy.

3. Methodology

See [supplementary material](#).

4. Conclusion

This study reports the first extensive phytochemical evaluation of *C. sundaicum* stem bark and discloses the discovery of a new xanthone, sundaixanthone A, together with six known xanthones. The notable antioxidant and antibacterial activities, supported by molecular docking that shows inhibition against PBP, spotlight the species as a promising source of therapeutic lead compounds against multidrug-resistant *A. baumannii*. These findings contribute to a better understanding of the chemical diversity and biological significance of the genus *Calophyllum*. Future work should focus on validating the *in vitro* mechanism of sundaixanthone A to confirm its PBP inhibitory potential.

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Author contributions

CRedit: **Mas Atikah Lizazman**: Conceptualization, Methodology, Writing – original draft, Writing – review & editing; **Jong Yi Mian Vivien**: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing; **Nor Hisam Zamakshhari**: Methodology, Supervision, Writing – original draft, Writing – review & editing; **Yiizamy Suffian**: Methodology, Software, Writing – original draft, Writing – review & editing; **Mohamad Izwan Bin Ismail**: Methodology, Writing – original draft, Writing – review & editing; **Enis Nadia Md Yusof**: Methodology, Writing – original draft, Writing – review & editing; **Nurr Maria Ulfa Binti Seruji**: Data curation, Methodology, Writing – original draft, Writing – review & editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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