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Phytochemical characterization of bioactive compounds extracted with different solvents from *Calophyllum inophyllum* flowers and activity against pathogenic bacteria

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ABSTRACT

Calophyllum inophyllum is used in traditional medicine to treat several diseases and conditions. Several studies have attempted to isolate useful compounds from various parts of this plant. However, the phytochemical constituents of *C. inophyllum* flower have not been extensively studied. This pioneering study focused on the chemical composition of *C. inophyllum* flower analyzed by gas chromatography-electron ionization/mass spectrometry (GC-EI/MS) and the antibacterial effects of *C. inophyllum* flower extracted with organic solvents. Phytochemical compounds were obtained from *C. inophyllum* flower via maceration with sequential extraction using hexane, ethyl acetate, and methanol, respectively. Phytochemical components of total phenolic (TP), total flavonoid (TF), and total saponin (TSC) contents were determined by colorimetric methods and each extract was found to be rich in phenolic, flavonoid, and saponin constituents. The antibacterial activities of the extracts were studied by disk diffusion method. The minimum inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) assays were used to address the potentials of extracts. All extracts were active against pathogenic bacteria at different concentrations, and were especially active against *Salmonella tyhpi*. In addition, the hexane extract exhibited the lowest MIC and MBC of 0.098 and 3.12 mg/mL, respectively, against *B. cereus* based on the antibacterial dilution method. The correlation analysis indicated a negative relationship between the flavonoid content and the inhibition zone of *Salmonella typhi*, with a significant value of $p < 0.05$. On the contrary, a positive relationship between the saponin content and the inhibition zone *Klebsiella pneumoniae*. These results showed that *C. inophyllum* flower extracts are rich of bioactive compounds such as phytol, eugenol, caryophyllene oxide, α-copaene, α-muurolene, β-caryophyllene, β-amysin, farnesol, palmitic acid, and cadinene derivatives.

1. Introduction

Folk wisdom regarding the use of herbal medicine has been passed down through generations. Some herbs exhibit specific healing qualities, which can be used to treat many symptoms. Researchers have extensively studied active biomolecules present in herbs. In medicinal plants, these biomolecules can be primary or secondary metabolites (Ghorbanpour et al., 2016a, 2106b; Roessner and Beckles, 2009). The rich biodiversity of Thailand provides a vast resource of medicinal plants, especially in mangrove zones. Plants found in mangroves can contain several primary and secondary metabolites, such as amino acids, vitamins, fatty acids, flavonoids, and phenolic acid, which are associated with important medicinal profiles. In Thai traditional medicine, the mangrove plant, *Calophyllum inophyllum*, known in English as mastwood among other names, is used to treat various ailments such as wound healing, arthritis and skin diseases.

C. inophyllum is a medicinal plant in the Calophyllaceae family. It dominantly grows in land–sea transitional ecosystems of tropical forest. It is a medium-sized tree up to 20 m in height with rough bark and white flowers. The flower, with the average size of 25 mm in width, occurs in racemose or paniculate inflorescences consisting of 4–15 flowers. The blooming is all-year-round. the fruit (the ballnut) appears as rounded and green drupe with 2–4 cm in diameter and a single large seed is located at the center. Previous research reported that the plant exhibits anticancer activities (Shanmugapriya et al., 2017) and antibacterial activities (Malarvizhi and Ramakrishnan, 2014). Several parts of the plant contain bioactive compounds such as flavonoids, alkaloids, steroids, terpenoids, and saponins (Hapsari

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et al., 2022; Mah et al., 2015; Susanto et al., 2019). In addition, antioxidant and antibacterial activities, due to the presence of phenolic substances, have been reported (Cassien et al., 2021; Saechan et al., 2021). The phenolic compounds were confirmed by the N—H and OH components in two mangrove plants, *Rhizophora apiculata* and *Rhizophora annamalayana* (Arulkumar et al., 2020). Their antioxidant activities involved functional groups that can eliminate radicals, reduce stress, and prevent diseases. There have been reports on bioactive components in extracts of *C. inophyllum* leaf, stem, bark, fruit, seed, root, and flower. Petroleum ether, methanol, ethanol, acetone, ethyl acetate, and water were among the solvents used for extraction (Hapsari et al., 2022; Kadir et al., 2015; Ojah et al., 2020; Sakthivel et al., 2019; Saravanan et al., 2011). Compounds such as phytol, linoleic acid, methylisostearate, and diphenylmethane extracted by methanol, may prevent incurable diseases such as common cold, cancer, asthma, and allergic diseases (Saravanan et al., 2015). Linoleic acid, methyl ester and hexadecanoic acid (palmitic acid) obtained from the slow pyrolysis of wood bark have found a wide range of applications in treating ulcers, wounds, and burns. As enzyme inhibitors, they have been used in the treatment of schizophrenia (Sakthivel et al., 2019). The hydrodistillation of *C. inophyllum* leaf, flower, seed, pod, and root yielded γ-terpinene which was used for its antioxidant and anti-inflammatory effects (Ojah et al., 2020; Wen et al., 2018). Different bioactive compounds can be obtained by extraction using different solvents. Gas chromatography-mass spectrometry (GC–MS) has been used for the evaluation of metabolites and volatile compounds in plant extracts, such as *Broussonetia luzonica* (Franelyne et al., 2016), *Avicennia marina* (Rozirwan et al., 2022), *Calophyllum inophyllum* (Saravanan et al., 2015) and *Dregea volubilis* (Singamoorthy et al., 2021).

Up to now, metabolite profiling by GC–MS has not been used to identify bioactive compounds obtained by solvent extraction of *C. inophyllum* flower using hexane, ethyl acetate, and methanol. To the best of our knowledge, this is the first report on the identification of bioactive compounds from *C. inophyllum* flower extracted with solvents of different polarities including hexane, ethyl acetate, and methanol. GC–MS analysis was used to identify the chemical composition of volatile bioactive compounds in the extracts and *in vitro* assays were used to determine their antibacterial activity.

2. Materials and methods

2.1. Plant collection

Flowers of *C. inophyllum* were collected during September 2016 from coastal region around Rajamangala University of Technology Srivijaya, Trang Province, Thailand. The plant material was identified and authenticated by the Department of National Parks, Wildlife and Plant Conservation, Thailand. A herbarium voucher was submitted as BKF. 194,811.

2.2. Plant extraction

The collected flowers were dried in the shade for a week. Five hundred grams of powdered flowers were extracted with 500 mL of hexane by maceration for five days. The extract was then filtered and concentrated at reduced pressure using a rotary evaporator at 45 °C. The residue was macerated further with ethyl acetate and methanol using the same procedure as for hexane. All extracts were stored in vials at 4 °C until analyzed.

2.3. Total phenolic compounds

The Folin-Ciocalteu colorimetric method, adopted from Vittaya et al. (2019), was used to determine the total phenolic content (TPC) in the extracts of *C. inophyllum* flower. The calibration curve was prepared using Gallic acid as the standard and the values were expressed in mg of gallic acid equivalent (GAE) per gram of crude extract. The calibration curve was linear, represented by a regression equation of $y = 0.0040x + 0.0086$ with R^2 of 0.9977.

2.4. Total flavonoid contents

The total flavonoid content (TFC) in the extracts of *C. inophyllum* flower was determined by colorimetric assay as described by Vittaya et al. (2020). Rutin was used as the standard for the calibration curve, expressed in mg of rutin equivalents (RU) per gram of crude extract. The linear calibration curve was represented by a regression equation of $y = 0.0016x + 0.0002$ with R^2 of 0.9992.

2.5. Total saponin contents

The total saponin content (TSC) of the extracts of *C. inophyllum* flower was determined according to the method reported by Senguttuvan et al. (2014). Briefly, about 0.2 mL of extract (1 mg/mL) was mixed with 0.5 mL of 0.8% (w/v) vanillin solution. Then 5 mL of 72% sulfuric acid was added. The mixture was kept for a minute before the incubation at room temperature for 10 min. The incubated mixture was rapidly cooled in ice water to room temperature. The absorbance of the mixture was measured at 560 nm using UV–vis spectroscopy (U-1800 spectrophotometer, Hitachi High-Tech Science Corp., Tokyo, Japan). Escin was used as the standard for preparing the calibration curve, expressed in mg of escin equivalents (EC) per gram of crude extract. The curve can be described by a regression equation of $y = 0.0007x + 0.0254$ with R^2 of 0.9918. The experiment was performed in triplicate and the obtained data were expressed as the means ± standard deviation.

2.6. Fourier transform infrared spectroscopy (FTIR) analysis

Using a mortar and pestle, about 1 mg of each extract of *C. inophyllum* flower was mixed with 3 mg of dry potassium bromide (KBr) to form a disk. All the IR spectra were recorded at 25 °C in the mid-infrared range (4000 – 400 cm^{-1}) using the BX PerkinElmer FTIR spectrophotometer (PerkinElmer, Massachusetts, USA.). Each spectrum was displayed in terms of absorbance of%T.

2.7. Gas chromatography-electron ionization/mass spectrometry (GC-EI/MS)

Each *C. inophyllum* flower extract was analyzed using the 7890 B GC gas chromatograph (Agilent Technologies, California, USA.) equipped with HP-5 ms capillary column (30 mm length × 0.25 mm I. d. × 0.25 μm film thickness). The analysis was carried out as follows. Briefly, 20 mg of crude extract were dissolved in 1 mL of solvent (either hexane, ethyl acetate, or methanol) and then shaken by vortex. The solution was sonicated for 2 min followed by filtration through a nylon membrane (pore size: 0.2 μm) to remove debris. One microliter of each sample was introduced via a split injector at a temperature of 250 °C with a split ratio of 10:1. The oven temperature program was 40 °C for 2 min, raised at 5 °C/min to 320 °C, and held for 10 min. The carrier gas was helium at a constant flow rate of 1 mL/min. The temperature of MSD transfer line was at 320 °C. The solvent delay was 5 min. Tandem mass spectra (700D MS, Agilent Technologies, California, USA.) were produced by electron ionization (EI) at 70 eV. The ion source temperature was at 230 °C. The mass spectrometer was operated in full scan mode from *m/z* 35–550. MassHunter software was used to control the GC–MS and data acquisition. The chemical constituents were identified after comparing the mass spectral configurations obtained with the available mass spectral database (WILEY10 and NIST14 libraries).

2.8. Determination of antibacterial activity

2.8.1. Bacterial strains and disk diffusion method

The test involved seven bacterial species. Three species were Gram positive bacteria; namely *Bacillus cereus* TISTR 036, *Staphylococcus aureus* TISTR 746, and *Staphylococcus epidermidis* TISTR 518. Four species were Gram negative bacteria; namely *Escherichia coli* TISTR 527, *Salmonella typhi* TISTR 2517, *Klebsiella pneumoniae* subsp. pneumoniae TISTR 1383, and *Pseudomonas aeruginosa* TISTR 2370. All bacteria were obtained from the National Center for Genetic Engineering and Biotechnology, Thailand. All extracts were screened three times for antibacterial activity using the paper disk diffusion method (NCCLS, 1993). Bacteria were incubated in Mueller Hinton broth at 35 °C for 2 − 6 h and the turbidity was measured at 0.5 McFarland standard (1.5×10^8 colony forming units/mL) at 625 nm, with the optical density (OD) between 0.08 and 0.10. The bacteria were swabbed over the surface of the media (Mueller Hinton agar) with a sterile cotton swab and allowed to solidify. Crude extracts were dissolved in dimethyl sulfoxide (negative control) to obtain stock solutions of 100 mg/mL and 10 μL of each sample was tested on a sterile Whatman No. 1 filter paper disk (6 mm in diameter). After incubation at 35 °C for 24 h, the zones of inhibition were measured in millimeters. Gentamycin was used as a positive control.

2.8.2. Estimation of minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

MICs of all extracts were evaluated using the standard method (CLSI, 2009) with a slight modification. Broth microdilution susceptibility procedure was performed using 96-well microplates. Briefly, 200 μL of the different extract concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391, 0.195, and 0.098 mg/mL) in 96-well culture plates with 0.85% sodium chloride were prepared by two-fold serial dilution with sterile Mueller Hinton Broth (MHB) in each well. The last well was the positive control which contained only the culture medium and bacterial suspension. Then, the inoculated microplates were incubated at 35 °C for 24 h. All tests were performed in triplicate. The MIC was considered as the lowest concentration of sample that inhibited the growth of the bacteria. The MBC was the lowest concentration of a crude plant extract that killed 99.9% of each bacteria used (0.08 − 0.1 OD or 1.5×10^8 colony forming units/mL).

2.9. Statistical analysis

The data were recorded from three replicates (n = 3) in each experiment and all the results were presented as means with standard deviation. One-Way Analysis of Variance (ANOVA) was carried out to compare the data and to further determine the statistically significant differences ($p < 0.05$). Correlations between the values for phytochemical composition (TPC, TFC, and TSC) in each extract with seven pathogenic bacteria were performed using Pearson's correlation coefficient.

Table 1
Yields of various extracts of *Calophyllum inophyllum*.

Plant	Part extracted	Solvent	Yield (g)	% Yield
C. inophyllum	Flower	Hexane	6.73	1.64
		Ethyl acetate	23.30	5.68
		Methanol	241.46	58.89

3. Results and discussion

3.1. Extraction yield

C. inophyllum flower was extracted using hexane, ethyl acetate, and methanol. Solvent polarity plays an important role on the solubility of phytochemical constituents (Ramalingam and Rajaram, 2018; Nalimanana et al., 2022), as do the structures of the phytochemical components. The polarity index of the solvents used in the present study can be arranged as follows: hexane (0.1) < ethyl acetate (4.4) < methanol (5.1) (Sadek, 2002). The highest yield was obtained with methanol extraction and the yield decreased with decreasing polarity of the extracting solvent (Table 1). The variation in the yields can be explained by the greater solubility of proteins and carbohydrates in methanol compared to hexane and ethyl acetate. These results confirmed the richness of polar substances in this plant. Our findings are in agreement with previous studies (Kadir et al., 2015; Mahmoudi et al., 2016), which reported that the maximum yield from *Temnocalyx obovatus* was obtained with absolute methanol, as well as our previous work (Vittaya et al., 2022).

3.2. Phenolic compounds, flavonoids and saponin contents

The TPC, TFC, and TSC of the three extracts of *C. inophyllum* flower ranged from 0.19 to 0.84 mg gallic acid equivalent per gram crude extract (mg GAE/g CE), 0.91 to 1.48 mg rutin equivalent per gram crude extract (mg Ru/g CE), and 2.99 to 5.05 mg escin equivalent per gram crude extract (mg ES/g CE), respectively (Table 2). The extraction of *C. inophyllum* flower using ethyl acetate provided the highest TPC and TFC, $p < 0.05$. Extraction using hexane resulted in the highest TSC. Phenolic and flavonoid compounds can be readily dissolved in a medium polarity solvent since they have similar polar properties. It is possible that the solubility of phytochemical compounds depends on the extraction solvent used and the degree of polymerization produced by the interaction of these compounds with other phytochemicals or vitamins (Naczk and Shahidi, 2004). TPC and TFC in each extract showed a positive trend with a correlation coefficient of 0.876, p = 0.002. On the other hand, TPC and TSC, and TFC and TSC showed negative trends with correlation coefficients of −0.794, p = 0.011 and −0.441, p = 0.235, respectively. The negative correlations between phenolic contents and flavonoid and saponin contents were attributed to the presence of other primary and secondary metabolites in each extract of this plant (Sajid et al., 2012).

Table 2
Total phenolic contents, total flavonoid contents, and total saponin contents of different extracts *Calophyllum inophyllum* flower.

Solvent	Total phenolic content* (mg GAE/g CE)	Total flavonoid content* (mg RU/g CE)	Total saponin content* (mg ES/g CE)
Hexane	0.19 ± 0.02^c	0.91 ± 0.18^b	5.05 ± 0.79^a
Ethyl acetate	0.84 ± 0.07^a	1.48 ± 0.11^a	3.14 ± 0.55^b
Methanol	0.57 ± 0.05^b	1.03 ± 0.07^b	2.99 ± 0.41^b

* Each value is expressed as mean ± standard deviation (SD) (n = 3). Values followed by a different letter superscript in the same column are significantly different ($p < 0.05$) and values having the same letters are not statistically significant ($p < 0.05$). GAE: gallic acid equivalent, CE: crude extract.

Table 3
Tentative functional group assignments of FTIR spectra of three different extracts of *Calophyllum inophyllum* flower.

Bond	Functional group	*C. inophyllum* (Wavenumber, cm^{-1})		
		Hexane	Ethyl acetate	Methanol
O–H stretch in alcohol	Alcohol, phenol	3438	3406	3420
sp^2 C–H Asymmetric stretch	Alkene	3140	3152	3155
N–H stretch	Benzene			
C–H stretch in $-CH_3$ and $-CH_2$	Alkane	2918	2928	2926
C≡N stretch	Alkyl nitriles	2362	2364	2364
C=O stretch	Carbonyl in carboxylic acid, ester, aldehyde, ketone Aromatic compound	1736	1708	–
C=O stretch in salts of carboxylic acid	Unsaturated aldehyde, ketone Conjugated and cyclic alkane	1604	1618	1610
N–H bend in primary amines	Amine			
N–H bend in secondary amines	Amine			
C–O stretch in salts of carboxylic acid	Aromatic	1400	1400	1400
C–F stretch	Methyl group/aliphatic alcohol			
N–H bend in aromatic amines	Aromatic, ester, alkyl aryl ether	–	1254	1254
C–N stretch in primary and secondary aliphatic amine	Aliphatic amine Alkyl halide			
C–O stretch in $-OCH_3$	Alcohol	1168	1096	1078
C–O stretch in secondary and tertiary alcohols	Alcohol			
C–F stretch	Halogen compounds			
O–H out of plane bend in alcohols	Alcohol	720	–	–
O–H out of plane bend in *cis*- RCH=CHR	Alkene			
C–Cl stretch	Alkyl halide	618	618	618

To identify the phytochemical contents of *C. inophyllum* flower, the separation of their components must first be considered based on the polarity of the solvent used, following the principle of "like dissolves like" (Reichardt and Welton, 2011). Hexane is a non-polar solvent with a polarity index of 0.1, which dissolves alkanes, fatty acids, sterols, alkaloids, and some terpenoids. Ethyl acetate, a medium polarity solvent with a polarity index of 4.4, extracts compounds with intermediate polarity such as flavonoids and quinones. Methanol is a high polarity solvent with a polarity index of 5.1 that can dissolve glycosides, polyphenols including tannins, and anthocyanins (Feng et al., 2019; Sadek, 2002). In addition, the phytochemical contents obtained with the three solvents might be influenced by factors such as growing area, mineral availability, time of harvest, and storage conditions before extraction (Figueiredo et al., 2008; Tiwari and Cummins, 2013). It is therefore possible that the solvent extraction method extracts bioactive compounds which might exhibit some unexpected biological characteristics.

3.3. *FTIR analysis of* C. inophyllum *flower extracts*

The FTIR spectra of *C. inophyllum* flower extracts showed similar profiles, except some differences between functional groups. All spectra showed characteristic absorption bands between 3500 and 600 cm^{-1} (Fig. 1S). Tentative assignments of FTIR spectral data are shown in Table 3. The results indicated that the hexane and ethyl acetate extracts of *C. inophyllum* flower had more functional groups than that of the methanol extract. The probable functional groups of compounds in these extracts are alkanes, alcohol, carboxylic acid, aromatics, esters, aliphatic amine, and alkyl halides (Gilbert, 2017). These results demonstrated the characteristic absorptions of OH, C=O, C–O, C–H, and C=C groups among others, and support the findings of previous works that mangrove plants are the rich sources of various secondary metabolites of phenolic and flavonoid compounds (N–H and OH molecules) (Agoramoorthy et al., 2008; Glasenapp et al., 2019; Rozirwan et al., 2022).

3.4. *Chemical compositions*

GC–MS is frequently used to identify the constituents of volatile compounds, long and branched chain hydrocarbons, alcohols, acids, and esters. This work is the first report of GC–MS profiling of hexane, ethyl acetate, and methanol extracts of *C. inophyllum* flower. The identified chemical compounds in terms of compound name, class, molecular formula, molecular weight, and% peak area were presented in Table 1S. A total of 97 different compounds were identified in the chromatograms of the three crude extracts (Fig. 1). The GC–MS profiles of hexane, ethyl acetate, and methanol contained signals of 54, 63, and 41 of the identified compounds, respectively. The compounds were mostly classified as terpenes, fatty acid, and fatty acid derivatives (Table 4). The major components of the hexane extract were sesquiterpenoid (24.04%) and triterpenoid (13.50%). The main component of the ethyl acetate extract was sesquiterpenoid (23.63%). On the other hand, the major component of the methanol extract was fatty acid and derivatives (27.30%). According to the literature survey, the determination of composition existing in *C. inophyllum* flower by GC–MS is rarely. There is only the extraction by hydrodistillation (Ojah et al., 2019, 2020) stated that the 25 identified compounds were found which is much less than that of our present work. The 63 identified compounds were found by hexane extraction. Sesquiterpenoid was the main component in hexane and ethyl acetate despite derivative of fatty acid in methanol. Bioactive compounds like α-copaene, α-muurolene, δ-cadinene, cedrene, and 1-hexadecanol were also detected in both hydrodistillation and solvent extraction. However, bioactive compounds of phytol, caryophyllene oxide, geranylgeraniol, β-amyrin, eugenol, farnesol, α-amyrin palmitate, and palmitic acid were only observed in this work. The majority of the phytochemicals identified are known to exhibit various important biological activities and many compounds exhibit pharmacological activities. The molecular structures of some of these pharmacologically active compounds are shown in Fig. 2.

The relatively greater antibacterial activity of the hexane extract in this study was correlated with the presence of a greater number of bioactive compounds including phytol, caryophyllene oxide, geranylgeraniol, β-amyrin, and eugenol. These compounds have been observed in many medicinal plant extracts. Phytol is an important diterpenoid that exhibits anticancer, antibacterial, and antioxidant activities (Saravanan et al., 2015; Song and Cho, 2015). Caryophyllene oxide is a sesquiterpenoid oxide commonly applied as an antifeedant, insecticide, and as a broad-spectrum antifungal in plant defense (Russo and Marcu, 2017). Geranylgeraniol is a potential antibacterial inhibitor of *Mycobacteruim tuberculosis* (Vik et al., 2007). The compound β-amyrin is known to possess analgesic and antimicrobial properties (Sundur et al., 2014).

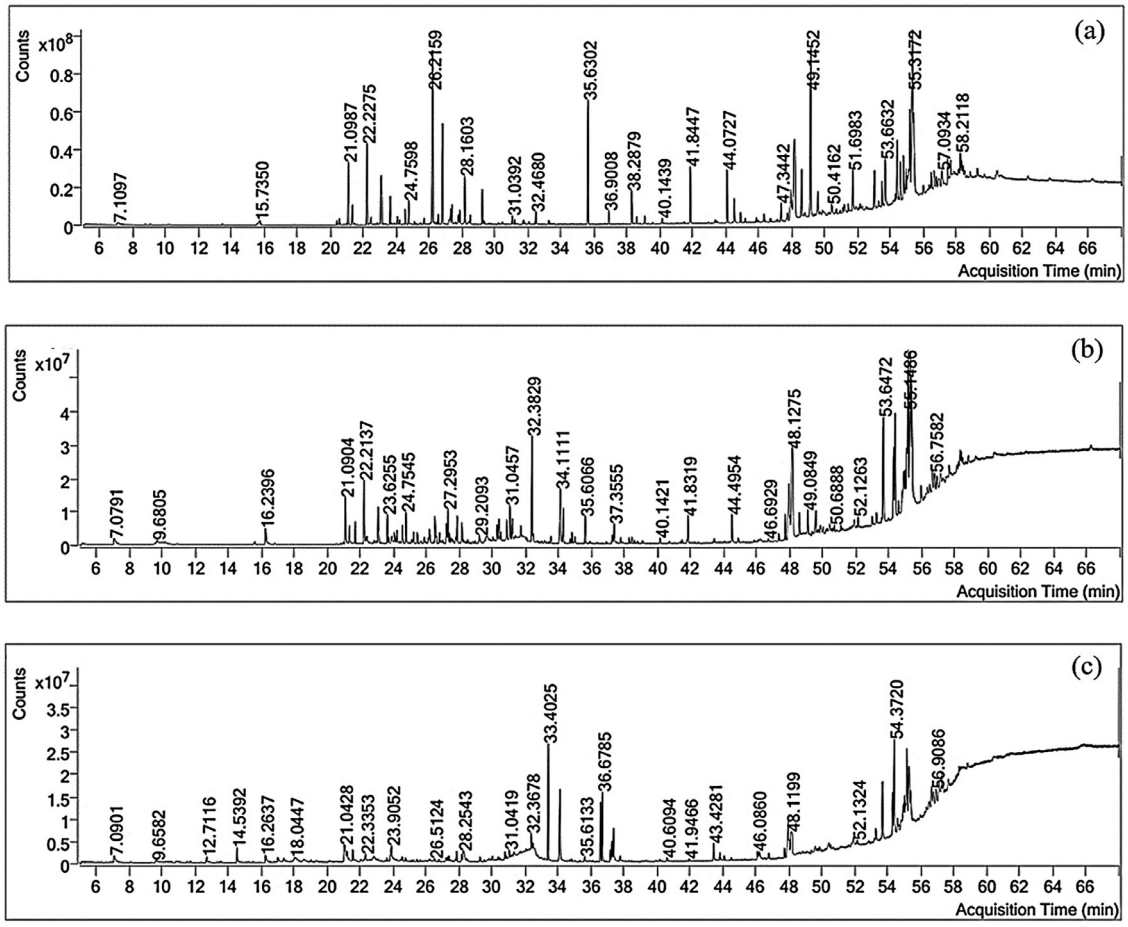

Fig. 1. GC–MS Chromatograms of hexane (a), ethyl acetate (b), and methanol (c) extracts of *Calophyllum inophyllum* flower.

Eugenol is an essential oil component providing excellent antimicrobial activity against fungi and a wide range of Gram positive and Gram negative bacteria (Marchese et al., 2017). Farnesol is found in the essential oils of various plants, exhibiting potent anti-inflammatory and anticancer effects, as well as alleviating allergic asthma (Jung et al., 2018). α-amyrin palmitate was regarded as an effective agent against arthritis (Aragão et al., 2008). Additionally, *n*-hexadecanoic acid (palmitic acid) and α-linolenic acid in ethyl acetate and methanol extracts are known to possess various biological activities. Palmitic acid is a saturated fatty acid found in almost all plant oils and microorganisms (Senthilkumar et al., 2015). For example, it was isolated from *Rhizophora mucronata* leaf and significant antibacterial activities were observed (Joel and Bhimba, 2010). α-Linolenic acid is an efficient antioxidant against oxidative DNA damage (Pal and Ghosh, 2012). α-copaene and α-muurolene in the hexane and ethyl acetate extracts were also reported to for antioxidant, antiparasitic, and antimicrobial activities (Karakaya et al., 2016; Mennai et al., 2021). Similarly, β-caryophyllene, γ and δ-cadinene in all three extracts are valuable bioactive compounds presenting anticancer, antioxidant, and antimicrobial activities (Dahham et al., 2015; Kundu et al., 2013; Mennai et al., 2021).

Table 4
Classification of identified phytochemicals in *Calophyllum inophyllum* flower extracts.

Class	*C. inophyllum* extracts (%)		
	Hexane	Ethyl acetate	Methanol
Number of identified compounds	54	63	41
Total identified compound contents	85.79	82.39	45.62
Diterpenoid	8.34	1.67	0.37
Monoterpenoid	-	1.35	-
Sesquiterpenoid	24.04	23.63	4.55
Triterpenoid	13.50	0.91	-
Quinone and hydroquinone lipid	0.32	0.66	-
Benzene and substituted derivative	2.56	4.31	3.80
Carbonyl compound	4.42	10.53	0.41
Cinnamic acid and derivative	-	0.37	1.61
Coumarin and derivative	4.59	11.49	-
Epoxide	4.03	0.96	0.38
Fatty acid and derivative	3.31	6.89	27.30
Glycerolipid	-	0.16	1.24
Organoheterocyclic compound	4.51	5.48	-
Organooxygen compound	-	1.25	-
Phenol	0.25	1.24	4.28
Pyran	-	-	1.12
Saturated hydrocarbon	11.01	4.49	0.56
Unsaturated hydrocarbon	1.78	1.63	-
Steroid	3.13	5.37	-

The GC–MS data confirmed that the selection of solvent with the appropriate polarity is one of the main factors affecting the extraction process. There is a possible interaction between each secondary metabolite and plant components such as proteins, lipids, and carbohydrates. These interactions can result in the formation of complexes that are quite difficult to dissolve. The polarity of the solvent used also affects the solubility of compounds. These results were in good agreement with the work of Wakeel et al. (2019), which showed that different parts of the woad plant, containing different levels of secondary metabolites with specific polarities, require extraction with specific solvents of suitable polarity. Solvent and solute polarity could be one of the reasons for the higher antimicrobial activity exhibited by the hexane extract of *C. inophyllum* flower compared to that of ethyl acetate and methanol extracts as stated earlier.

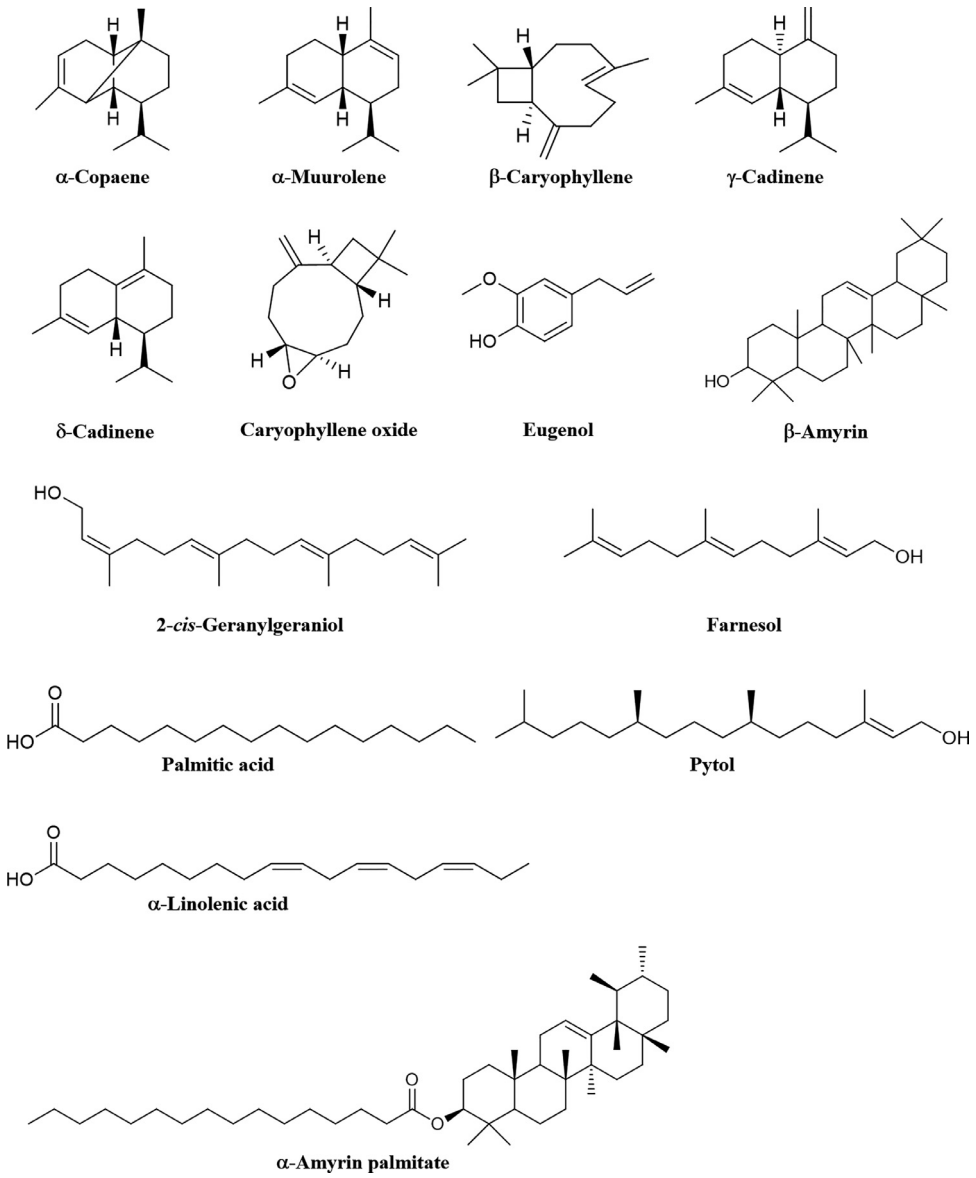

Fig. 2. The molecular structure of some compounds identified in hexane, ethyl acetate, and methanol extracts of *Calophyllum inophyllum* flower.

Table 5
Antibacterial activity of *Calophyllum inophyllum* flower extracts against seven pathogenic bacteria strains.

Solvent	Zone of inhibition diameter (mm)						
	Gram positive			Gram negative			
	B. cereus	*S. aureus*	*S. epidermidis*	*E. coli*	*S. typhi*	*K. pneumoniae*	*P. aeruginosa*
Hexane	15.07±1.03^{b}	14.95±0.29^{b}	8.48±0.51^{b}	8.54±0.51^{b}	16.63±0.79^{b}	8.44±0.31^{b}	13.92±3.18^{b}
Ethyl acetate	14.46±0.21^{b}	14.81±0.85^{b}	8.61±0.58^{b}	8.25±0.60^{b}	14.35±0.94^{c}	7.70±0.39^{c}	12.01±2.74^{b}
Methanol	14.09±0.75^{b}	13.66±0.22^{c}	8.58±0.30^{b}	7.68±0.65^{b}	16.62±0.35^{b}	6.85±0.25^{d}	12.55±2.18^{b}
Gentamycin	23.15±0.12^{a}	23.91±0.06^{a}	22.01±10.34^{a}	20.74±0.04^{a}	26.14±0.04^{a}	18.69±0.15^{a}	23.41±0.17^{a}

Data shown as means ± SD from triplicate analysis.
Different lowercase superscripts in a column denote significant ($p < 0.05$) differences in means ± SD values.

3.5. Antibacterial activity

Three different *C. inophyllum* flower extracts were evaluated for growth inhibitory activity against seven bacteria. Three strains were Gram positive bacteria (*B. cereus, S. aureus*, and *S. epidermidis*) and four were Gram negative bacteria (*E. coli, S. thyphi, K. pneumoniae*, and *P. aeruginosa*). Photographs of the results of the disk diffusion assay are included in Fig. 2S. All extracts showed antibacterial activity against all pathogenic bacteria. The antibacterial activity of the extracts against the Gram positive bacteria was similar to their antibacterial activity against the Gram negative bacteria, except for *K. pneumoniae* where the inhibition zones produced by hexane, ethyl acetate, and methanol exhibited significant differences ($p < 0.05$) (Table 5).

Fig. 3 shows the effects of extracting solvents of *C. inophyllum* flower on the growth of seven bacteria, indicating that hexane and ethyl acetate extracts strongly inhibited the growth of *S. typhi, B.*

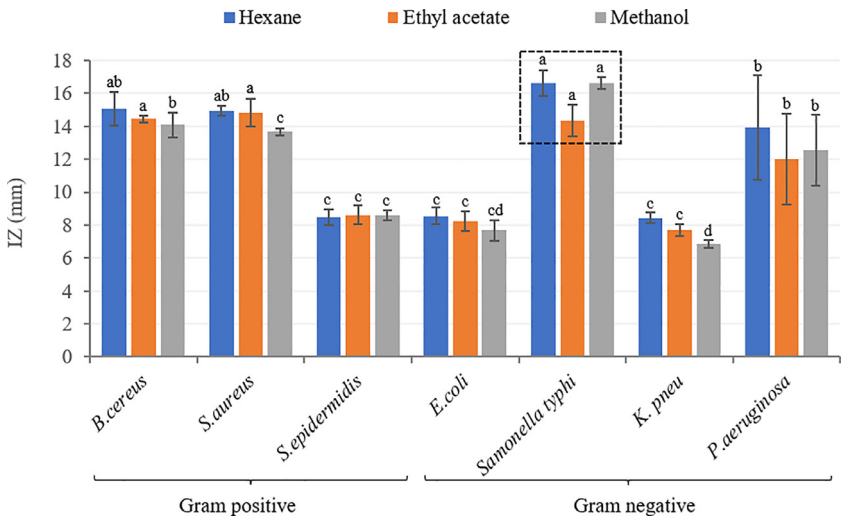

Fig. 3. Effects of extracting solvents of *Calophyllum inophyllum* flower on the growth of bacteria strains. Results are expressed as means ± SD (n = 3), significant ($p < 0.05$) differences. Different lowercase superscripts denote significant ($p < 0.05$) differences in means ± SD values.

cereus, and *S. aureus* ($p < 0.05$). Methanol extract was only strongly active against *S. typhi* ($p < 0.05$). Notably, all three extracts were most active against *S. typhi*. The stronger resistance of the Gram negative bacteria *E. coli* and *K. pneumoniae* to the extracts could be attributed to the complexity of the double membrane expressed by lipoprotein and lipopolysaccharide. The complexity of membrane structure presents a barrier to antibacterial substances compared to that of the single membrane structure of Gram positive bacteria *S. epidermidis* (Song and Cho, 2015; Yitayeh and Wassihun, 2022).

The MICs of the *C. inophyllum* flower extracts ranged from 0.098 to 12.5 mg/mL against Gram positive bacteria and from 6.25 to 50 mg/mL against Gram negative bacteria (Table 6). All extracts showed strong activity against *B. cereus* with low MIC values of < 0.098, 0.39 μg/mL and 0.098 μg/mL for hexane, ethyl acetate, and methanol, respectively. The mechanism of antibacterial activity is influenced by the molecular weight of the compounds involved. This affects the diffusion through agar, causing the difference in sensitivity exhibited by Gram positive and Gram negative species. Sensitivity differences are related to the chemical composition of the extracts (Russo and Marcu, 2017).

3.6. Relationship between the amount of TPC, TFC and TSC with antibacterial activities

Table 7 shows the correlation between the amount of phytochemical components with antibacterial activities. It appears that the phenolic content was positively correlated with flavonoid (r = 0.876; $p < 0.01$) and saponin (r = 0.794; $p < 0.05$) but no correlation was observed between flavonoid and saponin. The antibacterial activities against almost all bacteria strains were not significantly correlated with phenolic, flavonoid, and saponin contents, except for the flavonoid content against *S. typhi* (r = −0.777; $p < 0.05$). Saponin content was positively correlated with *K. pneumoniae* (r = 0.697; $p < 0.05$). It was possible that the discrepancies in antibacterial activity involved other specific phenolic, flavonoid, and saponin compounds in each extract or other phytochemical components, such as alkaloid, terpenoid, anthraquinone (Arulkumar et al., 2020; Singamoorthy et al., 2021). In this work, such classes of sesquiterpenoid, triterpenoid, fatty acid and derivatives, saturated hydrocarbon, carbonyl compound, coumarin and derivatives, and saturated hydrocarbon were founded in *C. inophyllum* flower which may be supported the antibacterial activity. Similarly, Vittaya et al. (2022) reported no significant or a negative correlation between the phenolic and flavonoid contents and their antibacterial activity against *Derris indica*.

4. Conclusion

To the best of our knowledge, this is the first report on the chemical profiles of bioactive compounds that can be extracted from *C. inophyllum* flower using hexane, ethyl acetate, and methanol. Depending on the type of solvent used, the yield of *C. inophyllum* flower extract was in an increasing order of methanol, ethyl acetate, and hexane. The total phenolic and flavonoid contents were significantly correlated and the maximum content of both were obtained from extraction using ethyl acetate. The highest content of saponin was observed when hexane was used in extraction. All extracts inhibited the growth of Gram positive and Gram negative bacteria, especially *S. typhi*. Moreover, the chemical composition in the extracts was rich in sesquiterpenoid, triterpenoid, fatty acid and fatty acid derivatives. The extracts could be developed for food and pharmaceutical applications. To realize the potential of this plant, further study is needed to investigate its toxicity and the environmentally friendly extraction method should be developed. LC-MS analysis can also be used as an alternative technique to study the correlation between chemical profiles and the biological properties of the investigated extracts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.sajb.2023.01.052.

Table 6
Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of *Calophyllum inophyllum* flower extracts.

Solvent	MIC and MBC (mg/mL)																				
	Gram positive									Gram negative											
	B. cereus			*S. aureus*			*S. epidermidis*			*E. coli*			*S. typhi*			*K. pneumoniae*			*P. aeruginosa*		
	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R	MIC	MBC	R
Hexane	< 0.098	3.12	<31.8	12.5	50	4	12.5	25	2	25	>50	>2	12.5	>100	8	50	>100	>2	25	>100	>4
Ethyl acetate	0.39	25	64	6.25	> 50	>8	6.25	25	4	6.25	50	8	12.5	>100	8	12.5	100	8	6.25	100	16
Methanol	0.098	3.12	31.8	6.25	6.25	1	6.25	50	8	12.50	>50	4	12.5	>100	8	25	>100	>4	12.5	>100	8

Values are expressed as means from quadruplicate determination ($n = 4$).
R values were calculated from MBC/MIC (R ⟨ 4.00 indicates bactericidal extract, R ⟩ 4.00 indicates bacteriostatic extract).

Table 7
Correlation coefficient (r) between phytochemical compositions and seven pathogenic bacteria of *Calophyllum inophyllum* flower.

Variables	TPC	TFC	TSC	*B. cereus*	*S. aureus*	*S. epidermidis*	*E. coli*	*S. typhi*	*K. pneu*	*P. aeruginosa*
TPC	1	0.876**	−0.794*	−0.371	−0.078	0.103	−0.178	−0.649	−0.551	−0.446
TFC		1	−0.441	−0.352	0.330	0.181	−0.181	−0.777*	−0.243	−0.607
TSC			1	0.198	0.458	−0.068	0.324	0.195	0.697*	0.265
B. cereus				1	0.188	−0.606	0.269	0.262	0.490	0.472
S. aureus					1	−0.107	0.539	−0.123	0.522	−0.259
S. epidermidis						1	0.313	−0.331	0.112	−0.419
E. coli							1	−0.132	0.531	−0.492
S. typhi								1	−0.124	0.375
K. pneu									1	0.375
P. aeruginosa										1

TPC = total phenolic content; TFC = total flavonoid content; TSC = total saponin content.
*, ** = significant different from 0 at * $p < 0.05$ and ** $p < 0.01$, respectively; ns = non-significant ($p > 0.05$). Data shown are mean ± SD. Pearson's correlation coefficient (r) between each chemical composition ($n = 9$) and bacteria. * $p < 0.05$, ** $p < 0.01$.

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