

Anti-oxidant and anti-bacterial activities of 11 *Calophyllum* species from Indonesia

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Abstract

Members of *Calophyllum* (Calophyllaceae) are commonly used as herbal medicine in Indonesia from the beginning of civilization. Due to their tremendous potential as a source for pharmaceutical industry, we selected 11 *Calophyllum* species for the current anti-oxidant and anti-bacterial studies, namely, *Calophyllum euryphyllum*, *C. bicolor*, *C. flavoramulum*, *C. incrassatum*, *C. lowei*, *C. macrophyllum*, *C. nodosum*, *C. soulattri*, *C. tetrapterum*, *C. teysmannii*, and *C. tomentosum*. The methods include *Calophyllum* stem bark extraction and separation into n-hexane, ethyl acetate, and butanol fractions, followed by the antioxidant analysis using DPPH (2,2-diphenyl-1-picrylhydrazyl radical scavenging) and the anti-bacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. The results showed that n-hexane fraction of *C. soulattri* exhibited the best anti-oxidant activity ($IC_{50} = 2.2 \mu\text{g}/\text{m}$). This study also showed that a xanthone compound isolated from *C. macrophyllum*, namely, 1,8 - dihydroxy-6 methoxy xanthone exhibited the anti-oxidant activity with $IC_{50} = 1.56 \text{ ppm}$.

Keywords: anti-bacterial, bioactive, *Calophyllum*, stem extract, xanthone

Introduction

Calophyllum (Indonesia: Nyamplung) is the largest genus in *Calophyllaceae* with about 190 species (Stevens 2006). Members of this genus are distributed mainly in South and Southeast Asia, but also in Eastern Africa, tropical America, Australia, Madagascar, the Pacific island, Atlantic forest, and Brazil. *Calophyllum* species are large hardwood and are generally grow in a mountain forest to coastal swamps and restricted to the hot and humid tropic (Noldin 2006). The genus *Calophyllum* is not only potential for its timber, but also for their medicinal properties such as xanthenes and coumarins to which have been potential as anti-viral, anti-tumor, cytotoxic, anti-malarial, anti-bacterial, anti-gastrointestinal, and anti-human immunodeficiency virus properties, anti-inflammatory, and ulcers (Iinuma 1994,1997 Yimdjo 2004, Xiao-HS *et al.* 2008, Cechinel *et al.* 2009, Li 2016, Sri 2006). The main parts used the leaves or stem barks, in the form of tea (Noldin 2006).

Prenylated xanthenes (Iinuma M, 1997) from *Calophyllum apetalum*, Caloxanthenes O and P (Dai Hao-Dai 2010) from *C.inophyllum*, and two xanthone (Linuma 1994) from *C. inophyllum*, also six xanthenes (Iinuma 1996) from *C. austroindicum* and then trioxxygenated diprenylated chromenxanthone (Dharmaratne 1997) from *C. moonii* have isolated, but they did not do bioassay activities. Four new coumarin (Li 2016) from *C. inophyllum*, coumarins

mammea type (Chilpa 2004) from *C. brasiliense*, and three isomeric pyranocoumarin (Vittal 1998) from *C. teysmannii* and minor coumarin (Cao-Shu-Geng 1998) from *C. teysmannii* and also triterpenoid (Li Y-Z 2010) from *C. inophyllum* have also isolated, but they have not done bioassay activities to four new coumarin from *C. inophyllum*, coumarins (Li Z 1 2016). The coumarin from *C. brasiliense* have cytotoxic activity to three cell lines e.i K562, U251 and PC3 human tumor cell lines (Chilpa 2004)

Zakarya in 2014 have isolated the anti-inflammatory activity compound from *C. inophyllum*. Polyisoprenylated acylphloroglucinols and a polyisoprenylated tetracyclic xanthone have isolated from the bark of *Calophyllum thorelii*, that compound have cytotoxic activity against to MCF-7 and Hela and NCL-H460 cell lines (Nguyen 2012). Anti-dyslipidemic and anti-oxidant activity compound have isolated from leaves *Calophyllum inophyllum* by (Prasad 2012). Many compound also isolated from *Calophyllum inophyllum* and that compound have antimicrobial activity and cytotoxic activity (Yimdjo 2004).

Previously, we isolated flavanol, and fatty acid from stem bark of *C. macrophyllum* collected from Mount Kerinci, Jambi. Flavanol have anti-diabetic activity (Frenki 2013). and fatty acid have cytotoxic activity (Abbas 2011), and anti-diabetic activity of flavanol (5,7,2',5'-tetrahydroxy flavan-3-ol) compound was investigated in-vitro and in-silico (Frenki 2018). In this study, we evaluated the anti-oxidant and anti-bacterial activities of different extracts of 11 *Calophyllum* species (*Calophyllum euryphyllum*, *C. bicolor*, *C. flavoramulum*, *C. incrassatum*, *C. lowei*, *C. macrophyllum*, *C. nodosum*, *C. soulattri*, *C. tetrapterum*, *C. teysmannii*, and *C. tomentosum*). These plants were collected from some several places in Indonesia.

The anti-oxidant activities of n-hexane, ethyl acetate and n-butanol extracts of 11 *Calophyllum* species were did by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and anti-bacterial activities of n-hexane, ethyl acetate extracts of 11 *Calophyllum* species were investigated by using *E. coli*, *S. aureus*, *B. subtilis*, and *P. aeruginosa*.

We have investigated the stem bark of 11 *Calophyllum* species in Indonesia, several species of *Calophyllum* traditionally been used as herbal medicine for various diseases such as vaginal discharge, rheumatism, scabies, ulcers, hair growers, malaria, bronchitis, gastrict, hepatic disturbance pain, inflammation, infection, varicose hemoroid and act as diuretic (Aminuddin 2016). Continuation of our current program concerning the isolation of anti-oxidant compounds from *C. macrophyllum*. The xanthone isolated from *C. macrophyllum* have anti-oxidant activities (free radical scavengers).

Many researcher have implicated oxygen radical and other oxygen-derived species such as superoxide radical anion, hydroxyl radical, singlet oxygen, hydrogen peroxyide and nitric oxide are produced in human cells and as important causative agents in wide range of disease and distorted in human body (Araújo 2012), Damage due to free radical is caused by ROS (reactive oxygen species) can easily damage of the cell membrane, they can attack lipid, protein, phospholipid, protein/enzymes, carbohydrates, DNA in cell by propagating a reaction cycle (Shahwar 2010), induce undesirable oxidation causing, DNA damage and cell death.

Antioxidant compound can terminate or retard the oxidation process by scavenging free radical, chelating free catalytic metals and nascent oxygen from environmental. On the other hand, many bacteria could also depend on these nascent oxygen for survival in any given environmental (Shahwar 2010),

Medicinal plant as anti-oxidant in reducing free radical-induced tissue injure. Many authors explained that anti-oxidant activity of plants is due to their phenolic/polyphenol compound. Xanthone from *C. macrophyllum* plant can be choosed and used as a source of natural antioxidant because xanthone contains phenolic compound, such as donating of a

hydrogen atom to DPPH radical and binding affinities of *Calophyllum* extracts and xanthone to have inhibitory activity of bacterial.

Materials and methods

Samples

Stem bark samples of 11 *Calophyllum* species were used in this study (*C. euryphyllum*, *C. bicolor*, *C. flavoramulum*, *C. incrassatum*, *C. lowei*, *C. macrophyllum*, *C. nodosum*, *C. soulattri*, *C. tetrapterum*, *C. teysmannii*, and *C. tomentosum*). These species were collected from several locations in Indonesia such as Bulungan Research forest, Palangkaraya, Central Kalimantan (*C. euryphyllum*, *C. bicolor*, *C. flavoramulum*, *C. lowei*, *C. nodosum*, *C. teysmannii*, and *C. tomentosum*); Mount Kerinci, Jambi Province (*C. macrophyllum*), Palalawan district, Riau province (*C. tetrapterum*), Jayapura, Papua (*C. incrassatum*, *C. soulattri*).

Bioactive compound isolation

Calophyllum stem barks were dried and crushed. These materials were further macerated with 70% ethanol, and then partitioned with n-hexane, ethyl acetate (EtOAc), or n-butanol. Each solvent was evaporated to obtain a n-hexane, ethyl acetate, and n-butanol extracts (Table 1). A hexane extract of *C. macrophyllum* was separated by chromatographic column with silica as stationary phase, and a mixture of n-hexane- ethyl acetate as the mobile phase with gradually raised polarity system. The resultant columns were monitored with TLC, the same fraction was combined, then reconstituted with a chromatographic column. The recovered compound was recrystallized by several solvents, to obtain a xanthone compound. The obtained crystals were analysed by LC-MS to determine the molecular weight. The compound was further elucidated by using NMR 1D and 2D (2D JNM-ECA-500), UV-VIS (Hitachi U-2000), and FTIR (IRPrestige-21, Shimadzu).

Anti-oxidant activity assay

The anti-oxidant activity assay was performed by using 1,1-diprenyl-2-picrylhydrazyl reagent with an initial sample concentration at 1000 ppm (2 mg/2 mL). For the anti-oxidant assay, the concentration of the sample was made to 25, 50, 100, and 200 ppm (w/v) in a methanol added with a DPPH, and then was incubated at 37°C for 30 min. Absorbance was detected at 517 nm. The effect of extracts resistance on the free radical 1,1-diprenyl-2-picrylhydrazyl can be observed with the colour change of 1,1-diprenyl-2-picrylhydrazyl from violet to yellow. When the tested compound was active as an anti-oxidant, the colour changes to yellow. This indicates the occurrence of free radical capture by the tested xanthone compound (Hanato *et al.* 1988).

$$\% \text{ inhibition} = \frac{\text{Absorbance}_{517} \text{ control} - \text{Absorbance}_{517} \text{ sample}}{\text{Absorbance control}_{517}} \times 100\%$$

DPPH scavenging activities of various concentration of the most effective extract were assessed to determine concentration that causes 50% of inhibition (IC₅₀).

Anti-bacterial activity assay

The agar-well diffusion method was employed in the anti-bacterial activities assay. n-hexane and ethyl acetate extracts of *C. soulattri*, *C. tetrapterum*, and *C. macrophyllum* were tested for the anti-bacterial activities. Selected pathogenic bacteria (*E. coli*, *S. aureus*, *B. subtilis*, and *P. aeruginosa*) were grown on Muller-Hinton agar medium (pH 7.3). A

suspension of each bacterium (1.45×10^6 CFU/mL) were streaked on the surface of the medium using sterile cotton swab to ensure the confluent growth of the bacteria. Agar wells (6 mm in diameter) were made in the medium, and then about 100 μ L of the extracts were delivered into each hole. All plates were incubated at 37°C for 24 hours, and the growth-inhibition zones were measured.

Results

Anti-oxidant activities of *Calophyllum* extracts

The stem bark of all *Calophyllum* species collected in Indonesia was macerated in 70% ethanol. All samples were successively partitioned with *n*-hexane, ethyl acetate, and *n*-butanol. Almost all *Calophyllum* extracts showed anti-oxidant activity, except the hexane extract of *C. nodosum* ($IC_{50} = > 500$ μ g/mL (Table 1).

Table 1. Partitioned results and anti-oxidant activities of all *Calophyllum* stem barks extracts

No.	<i>Calophyllum</i> species (stem bark weight)	Extract	Yield (g)	DPPH scavenging (IC_{50})* (μ g/mL)
1	<i>C. bicolor</i> (2.5 kg)	<i>n</i> - hexane	40	387.3
		Ethyl acetate	231.7	5.1
		<i>n</i> -butanol	96.7	8.1
2	<i>C. euryphyllum</i> (2.8 kg)	<i>n</i> - hexane	72.9	130.5
		Ethyl acetate	150.9	23.6
		<i>n</i> -butanol	75.0	14.9
3	<i>C. lowei</i> (2.0 kg)	<i>n</i> - hexane	160.8	393.1
		Ethyl acetate	17.4	14.1
		<i>n</i> -butanol	60.0	6.9
4	<i>C. teysmannii</i> (1.12 kg)	<i>n</i> - hexane	146.5	72.9
		Ethyl acetate	39.3	150.9
		<i>n</i> -butanol	165.3	75.0
5	<i>C. tetrapterum</i> (2.3 kg)	<i>n</i> - hexane	206.7	65.6
		Ethyl acetate	22.0	212.8
		<i>n</i> -butanol	311.4	11.4
6	<i>C. incrassatum</i> (2.1 kg)	<i>n</i> - hexane	146.5	407.4
		Ethyl acetate	139.0	5.9
		<i>n</i> -butanol	165.3	52.0
7	<i>C. nodosum</i> (3.3 kg)	<i>n</i> - hexane	104.9	>500
		Ethyl acetate	92.9	214.0
		<i>n</i> -butanol	152.9	4.5
8	<i>C. macrophyllum</i> 5 kg)	<i>n</i> - hexane	5. 0	407.4
		Ethyl acetate	59.5	54.4
		<i>n</i> -butanol	47.8	113.9
9	<i>C. flavoramulum</i> (3.2 kg)	<i>n</i> - hexane	79.4	193.2
		Ethyl acetate	43.8	4.0
		<i>n</i> -butanol	77.3	54.8
10	<i>C. tomentosum</i> (1.7kg)	<i>n</i> - hexane	49.6	206.0
		Ethyl acetate	49.1	67.1
		<i>n</i> -butanol	78.1	11.6

No.	<i>Calophyllum</i> species (stem bark weight)	Extract	Yield (g)	DPPH scavenging (IC ₅₀)* (µg/mL)
11	<i>C. soulattri</i> (1.8 kg)	n- hexane	50.5	2.2
		Ethyl acetate	86.3	30.1
		n-butanol	75.1	24.1
12	Isolated compound from <i>C. macrophyllum</i>	1,8 - dihydroxy- 6 methoxy xanthone	-	1.56

*(IC₅₀) < 50 µg/mL = very active, (IC₅₀) 51 - 100 µg/mL = active, (IC₅₀) 101 – 500 µg/mL = less active, (IC₅₀) > 500 µg/mL = not active

This study showed that the majority of *Calophyllum* extracts exhibited high DPPH scavenging activity, except hexane extracts of *C. bicolor*, *C. lowei*, *C. incrassatum*, *C. nodosum*, and *C. macrophyllum*. Among them, *C. soulattri* hexane extract showed highest anti-oxidant activity with IC₅₀ = 2.2 µg/mL, followed by the ethyl acetate extracts of *C. flavoramulum* (IC₅₀ = 4 µg/mL), *C. bicolor* (IC₅₀ = 5.1 µg/mL), *C. incrassatum* (IC₅₀ 5.9 µg/mL), and butanol extracts of *C. nodosum* (IC₅₀ 4.5 µg/mL) and *C. bicolor* (IC₅₀ 8.1 µg/mL). In addition, the ethyl acetate and butanol extracts of *C. bicolor*, *C. euryphyllum*, and *C. lowei* exhibited higher anti-oxidant activity than the hexane fraction. A xanthone compound isolated from *C. macrophyllum* exhibited a high anti-oxidant activity (IC₅₀ = 1.56 µg/mL) (Table 1).

Anti-bacterial activities of *Calophyllum* extracts

Minimum inhibition concentration (MIC) for five *Calophyllum* species was shown in Table 2. Five *Calophyllum* species fraction exhibited moderate to high anti-bacterial activities. This study indicated that among five species of *Calophyllum*, hexane fraction of *C. macrophyllum* showed the highest anti-bacterial activities compared to the remaining *Calophyllum* extracts.

Table 2. Minimum inhibition concentration for all extract of five *Calophyllum* species

Fraction	MIC (Minimum inhibition concentration) (µg / mL)			
	<i>E. coli</i>	<i>S aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>
<i>C. tetrapterum</i> Hexane fraction	1.292	1.264	1.292	1.505
<i>C. tetrapterum</i> Ethyl acetate fraction	-	-	-	-
<i>C. incrassatum</i> Hexane fraction	1.281	1.077	1.175	1.228
<i>C. incrassatum</i> Ethyl acetate fraction	1.264	1.260	2.845	1.281
<i>C. macrophyllum</i> Hexane fraction	1.024	0.979	0.925	0.954
<i>C. macrophyllum</i> Ethyl acetate fraction	1.162	1.176	1.062	1.418
<i>C. tomentosum</i> Hexane fraction	1.264	1.260	2.845	1.281
<i>C. tomentosum</i> Ethyl acetate fraction	1.315	12.281	1.192	2.010

Fraction	MIC (Minimum inhibition concentration) ($\mu\text{g} / \text{mL}$)			
	<i>E. coli</i>	<i>S aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>
<i>C. soulattri</i> Hexane fraction	1.315	1.152	1.085	1.077
<i>C. soulattri</i> Ethyl acetate fraction	1.085	1.181	1.033	1.352

The anti-bacterial activity of *C. soulattri* hexane fraction, *C. macrophyllum* ethyl acetate fraction, and *C. tetrapterum* ethyl acetate fraction were expressed in the diameter of the inhibition zone (Fig. 1). Growth diameter was obtained by disc diffusion method at different concentration of samples.

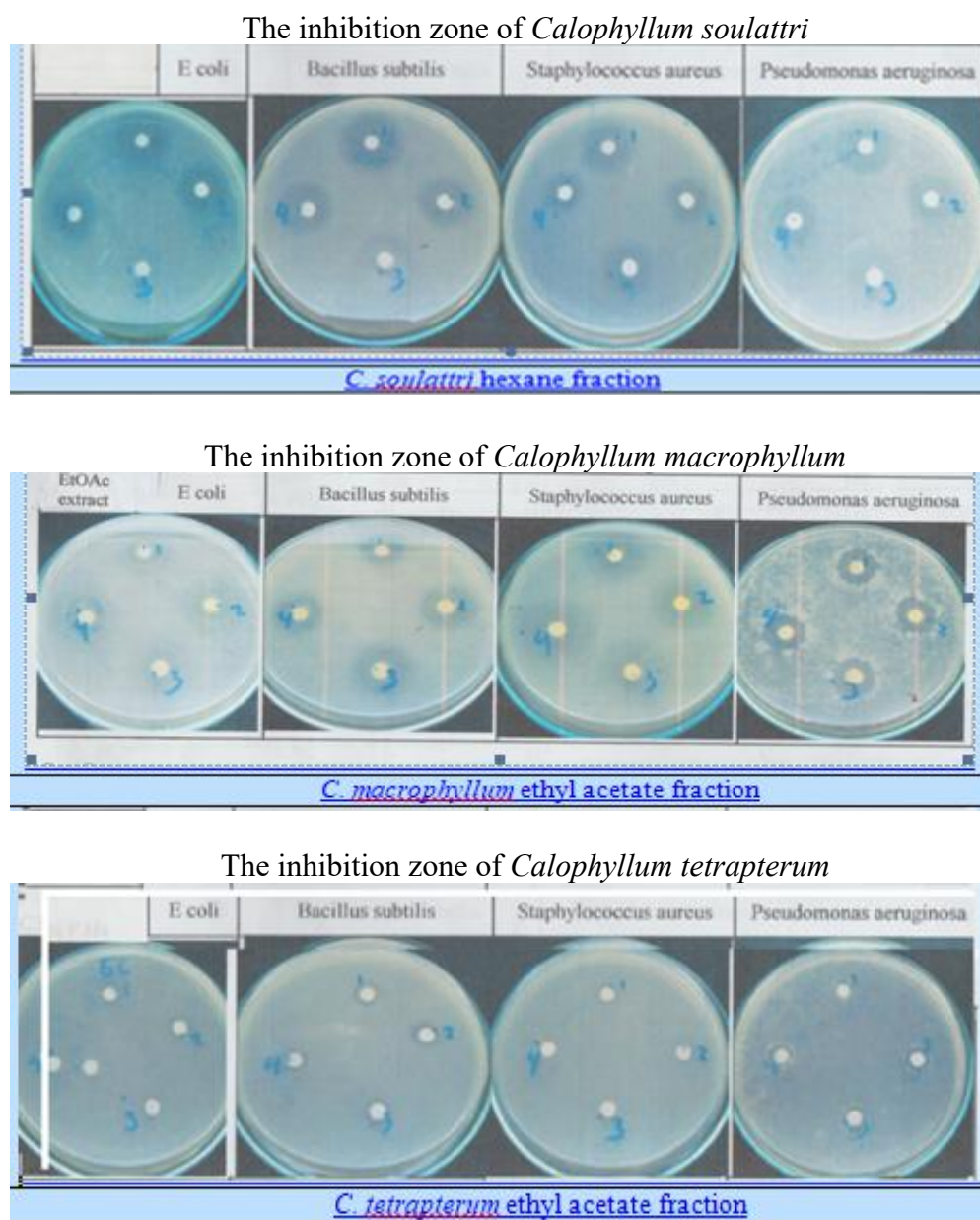


Figure 1. The diameter of zone inhibition some samples to organisms.

Antibacterial activity result of the 11 *Calophyllum* are shown in Table 2. MIC value range from 0.925 to 12. 281 $\mu\text{g/mL}$ were obtained for 11 *Calophyllum* species and against *E. coli* 1.024-1.315 $\mu\text{g/mL}$, and 0.979-12.281 $\mu\text{g/mL}$ for *S. aureus*, 0.925-2.845 $\mu\text{g/mL}$ for *B. subtilis* and 0.954- 2.010 for *P. aeruginosa*.

The IR spectrum illustrates strong band the presence free hydroxyl (OH^-) at ν max 3456 cm^{-1} , C-H stretching aromatic at 3157 cm^{-1} and the OH^- group conjugated to the carbonyl group at ν max 1697. There are four sharp absorption that occur in pairs 1622; 1519; 1469; and 1440 characteristic for C=C stretching aromatic ring. The presence of C-H scissoring at ν max 1384.89 and =C-H out-of-plan bending at 900-690 cm^{-1} . It supports that the compound obtained has a xanthone nucleus with two OH^- groups and one methoxyl (OCH_3) group, OH^- groups conjugated to C=O group (Fig. 2). The UV spectrum exhibited four maxim absorbent at lambda 327.5:248.0:232.0 and 202.0 nm.

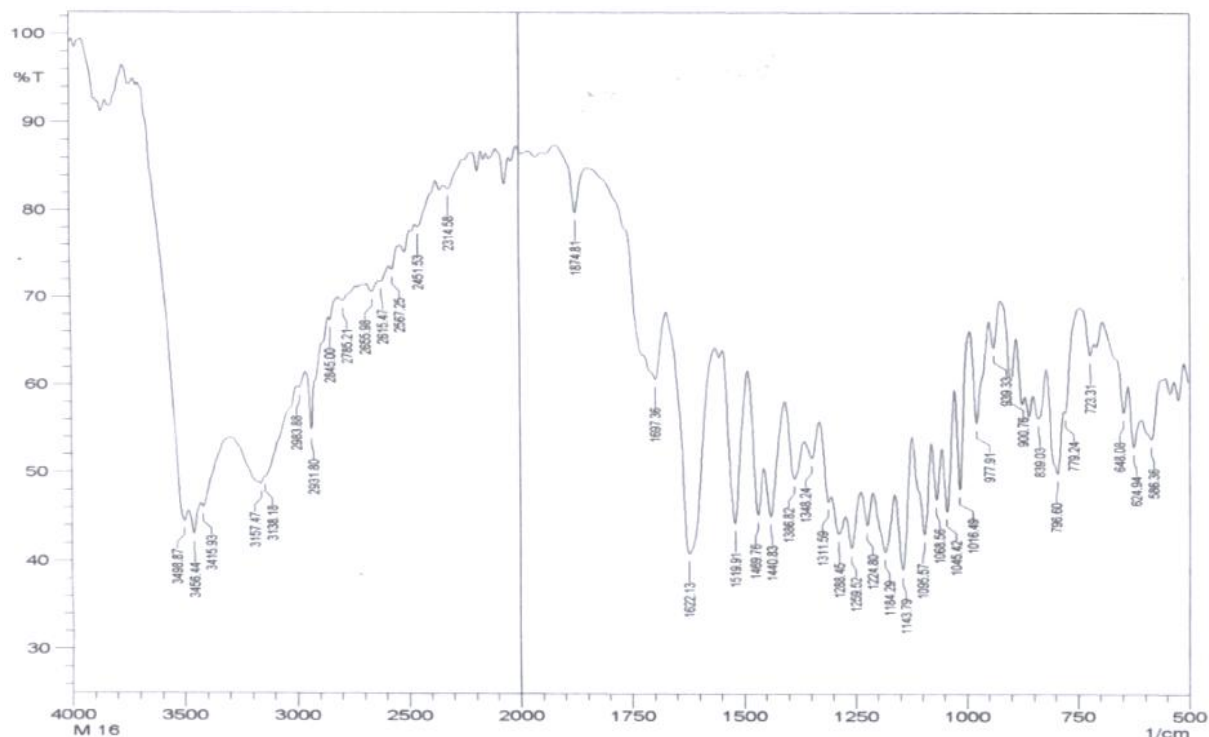


Figure 2. Infra-red (IR) spectrum of isolated compound from *C. macrophyllum*

In this study, the isolated xanthone compound was determined of consisting of a methoxy (O-CH_3) group. Xanthone compounds provide a positive $\text{FeCl}_3/\text{MeOH}$ test reagents (blue spot), it is indicating the present of the OH^- group. Mass spectrum showing the molecular ions ($\text{M} + 1$)⁺ at m/z 385.241 of which indicates the xanthone molecular weight was 384.241 with the molecular structure of $\text{C}_{23}\text{H}_{28}\text{O}_5$. The $^1\text{H NMR}$ spectrum showed the presence of two ortho-coupled protons [6.87 and 6.77 (1H each, d J 8.5 Hz)], presence one proton at 6.78 (1H, dd J 8.5 6.75 Hz), and one proton aromatic singlet at δ 6.52 (1H.s). The $^1\text{H NMR}$ analysis showed the existence of one methoxyl group at δ 4.04 (Fig. 3).

In the HMBC spectrum, the proton group δH of 6.87 ppm caused four cross peaks with four aromatic carbons at δC 157.9 (C-1), δC 115.6(C-3), δC 108.7(C-4) and δC 157.9(C-9a), and a proton at δH of 6.78 ppm caused four aromatic carbons at δC 157.9 (C-1), δC 110.8 (C-2), δC 108.7(C-4) and 153.5 (C-4a), and a proton δH of 6.77 ppm caused cross peaks with four aromatic carbons at δC 110.8 (C-2) and δC 115.6(C-3) and δC 153.5 (4a) and 107.1 (C-9a), and a proton aromatic at δH 6.52 ppm singlet caused cross peaks with four aromatic carbons at δC 122.5 (C-5), δC 136.9(C-6), δC 157.1 (C-8) and δC 150.6 (C-10a).

One aliphatic proton δ_H 1.58 ppm caused cross peaks with five carbons at δ_C 39.8 (C-11), δ_C 55.3 (C-12) and δ_C 29.8 (C-14, C-15), and δ_C 122.5 (C-5) respectively. One methoxyl group δ_H 4.04 ppm (3H, s) caused cross peaks with five carbons at δ_C 122.5 (C-5), δ_C 136.9 (C-6), and δ_C 100.0 (C-7) (Fig. 3). The HMBC data also showed that one OH⁻ group (δ_H 12.2) bound to C-1 at xanthone moiety, a proton from OH⁻ group (δ_H 12.2) caused cross peaks with three aromatic carbons at δ_C 110.8 (C-2), δ_C 202.1 (C-9) and δ_C 107.1 C-9a) and one OH⁻ group (δ_H 11.87) bound to C-8 at xanthone moiety, a proton from OH⁻ group (δ_H 11.87) caused cross peaks with three aromatic carbons at δ_C 100.0 (C-7), δ_C 202.1 (C-9), and δ_C 123.1 (C-8a). The substitution of hexyl group at C-5 in xanthone moiety was proven by HMBC data, a correlation between the proton methyl doublet δ_H 0.97 ppm (6H, *d J* 4.9) correlated to the carbon with δ_C 38.9 (C-11), δ_C 5,3 (C-12) and 49.3 (C-13), and one methyl singlet δ_H 0.93 ppm (3H, *s*) correlated to the carbon with δ_C 38.9 (C-11), δ_C 5,3 (C-12) and 49.3 (C-13), and one methyl singlet δ_H 0.82 ppm (3H, *s*) also correlated to the carbon with δ_C 38.9 (C-11), δ_C 5,3 (C-12) and 49.3 (C-13) (Fig. 4, Table 3).

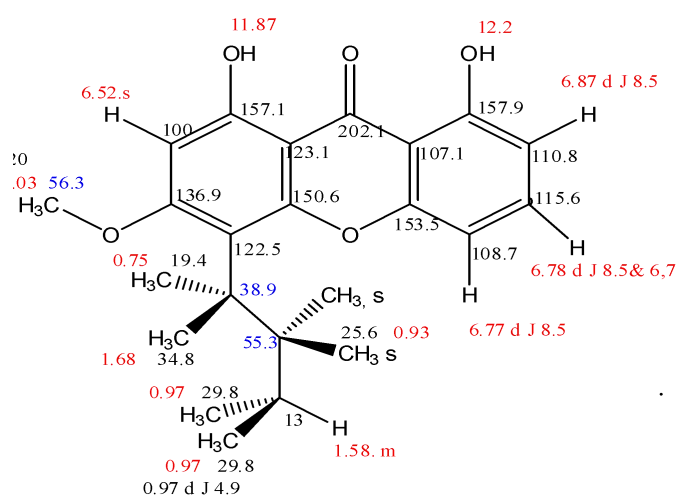


Figure 3. ^1H NMR and ^{13}C NMR spectrum values of the isolated compound from *C. macrophyllum*

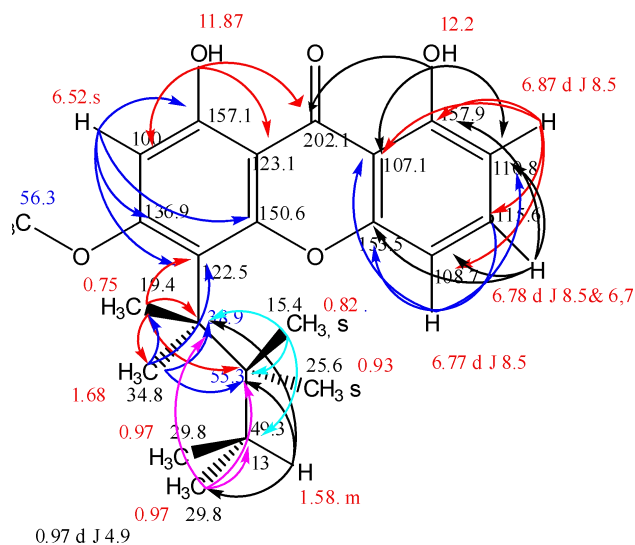
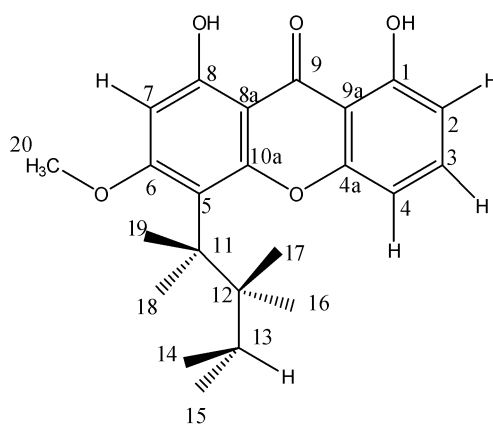


Figure 4. HMBC spectrum value of isolated compound from *C. macrophyllum*

Table 3. ^1H , ^{13}C -NMR, HMQC, and HMBC spectral data of the isolated compound from *C. macrophyllum*

No.	C	Chemical shifts (δ , ppm)	HMBC correlation
1	157.9 C-OH	12.2	C-2, C-9. C-9a
2	110.8	6.87 (1H,d J 8.5 Hz)	C-1, C-3, C-4, C-9
3	115.6	6.78 (1H,dd J 8.5, 6.75 Hz)	C-1, C-2, C-4, C-4a, C-9a
4	108.7	6.77 (1H, d J 8.5)	C-2, C-3, C-4a, C-9a
5	122.5	-	-
6	136.9	-	-
7	100.0	6.52 (1H,s)	C5, C-6, C-8. C-10a
8	157.1 C-OH	11.87	C-7, C-8a, C-9
9	202.1. C=O	-	-
4a	153.5	-	-
8a	123.1	-	-
9a	108.1	-	-
10a	150.6	-	-
11	29.8	-	-
12	55.3	-	-
13	49.2	1.54 (1H, m)	C-11, 12, C-14/C-15
14, 15	29.8	0.97 (6H, d J 4.9)	C-11, 12, C-13
16	25.6	0.93 (3H, s)	C11, C-12, C-13, C-17
17	15.4	0.82 (3H, s)	C11, C-12, C-13, C-16
18	29.8	1.68 (3H, s)	C-5,C11, C-12, C-19
19	19.4	0.75 (3H, s)	C-5,C11, C-12, C-18
20	56.3 (OCH ₃)	4.03 (3H, s)	C-5, C-6, C-7

Based on the basic spectroscopic analysis by using ^1H -NMR, ^{13}C -NMR, NMR-1D and 2D (HMBC, HMQC, DEEP), IR, melting point, and molecular weight confirmation by LC-MS, the xanthone was determined containing substitution on C-1 C-8 as hydroxyl groups, and methoxyl group at C-6, and therefore it was named as 1,8-dihydroxy-6-methoxyl xanthone with the molecular formula of $\text{C}_{23}\text{H}_{28}\text{O}_5$ BW = 384.241. The 1,8 - dihydroxy-6-methoxy xanthone compound showed the highest anti-oxidant activity with $\text{IC}_{50} = 1.56$ $\mu\text{g}/\text{mL}$ (Fig. 5).



$\text{C}_{23}\text{H}_{28}\text{O}_5 = \text{BM } 360.24$

Figure 5. Structure of xanthone isolated from *C. macrophyllum*

Discussion

In this study, we isolated bioactive xanthone compound from *C. macrophyllum* using extracting solvents with different polarities. The hexane extract of the *C. macrophyllum* give the most inhibitory effect in anti-bacterial assay (MIC 1.024; 0.979; 0.925; 0.954 µg/mL for *E. coli*, *S. aureus*, *B. subtilis*, and *P. aeruginosa*) respectively. The hexane extract of this *C. macrophyllum* plant was subsequently fractionated by column chromatograph eluting by *n*-hexane: ethyl acetate with gradient polarity system. In this study, we found 1.8-dihydroxy-6-methoxy xanthone. (Table 3, Figure 3, 4, 5) involved anti-oxidant activity,

1.8-dihydroxy-6-methoxy xanthone is one type of xanthone compound identified from *C. macrophyllum* hexane extract. In line with this study. Yimdjo 2004 reported various xanthenes to have anti-microbial activity. Dharmaratne (1999) published anti-microbial activity of xanthenes from *Calophyllum* species, against Methicillin-resistant *Staphylococcus aureus* (MRSA). Adewuji (2014) isolated antibacterial activity of acetone extracts from the seed oils of *Calophyllum inophyllum*.

From the structure of xanthone and binding affinities of xanthone acting to bacterial inhibition. Lin 2015 *et al* reported that generally, hydrophobic interaction was essential in the binding of xanthone/flavonoid to antibacterial activity. The planar structure and double bond C₁-C₂-C₃ of xanthone are advantageous for antibacterial activity and antioxidant activity. Regarding antioxidant activity, xanthone showed the highest radical scavenging activities in DPPH assay. Similar to the results, Jamilah (2011) reported that fatty acid from *C. macrophyllum* have been reported to have antioxidant activity.

Conflict of Interest

The authors state no conflict of interest from this manuscript.

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