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Antibacterial Test and Isolation of Xanthenes from Pericarps Mangosteen (*Garcinia mangostana* L.) using Calcium Oxide (CaO) as a Vacuum Liquid Chromatography (VLC) Stationary Phase

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Abstract: Xanthenes are one of the biggest classes of compounds in natural product chemistry. Xanthenes have been isolated from pericarp mangosteen (*Garcinia mangostana* L.) and then purified with VLC using calcium oxide (CaO) as the stationary phase and eluted using n-hexane: EtOAc of increasing polarity. Extraction of the pericarp mangosteen using the maceration method with acetone as the solvent. The isolation of xanthenes was carried out through various chromatography techniques, such as vacuum liquid chromatography (VLC), gravitational column chromatography (GCC), and thin layer chromatography (TLC). The molecular structure of the compound was identified based on spectroscopy data, including IR, 1D NMR (¹H and ¹³C), and 2D NMR (HSQC and HMBC). Based on the analysis of spectrum data, the isolated compound is α-mangostin. The acetone extract and α-mangostin were also examined for antibacterial activity against Gram positive (+) *Staphylococcus aureus* (*S. aureus*) ATCC 25923 and Gram negative (-) *Escherichia coli* (*E. coli*) ATCC 25922. The largest inhibition zone was shown by α-mangostin against *S. aureus* (12.16 mm) more than *E. coli* (12 mm).

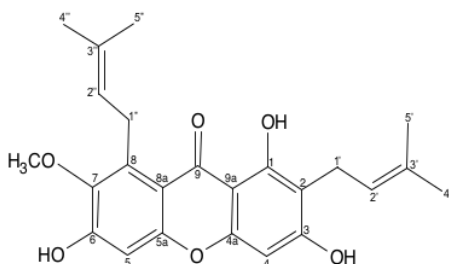
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INTRODUCTION

Xanthenes are one of the major compounds in *Garcinia*. They are secondary metabolites that commonly occur in higher plant families, fungi, lichen, and bacteria [1]. They have a variety of health-promoting properties, including antibacterial, anticarcinogenic, antioxidant, and antidiabetic properties [2]. Xanthenes have been isolated from pericarp mangosteen using various methods. Xanthenes are commonly isolated by VLC on silica gel as a stationary phase and using different solvent mixtures with increasing polarity [3].

Various studies for the stationary phase from different isolation methods of xanthenes have been carried out. Alumina is one of the stationary phases for xanthone isolation. Alumina was effective to separate 1,5-dihydroxyxanthone [4]. Polyamide columns are frequently applied for the separation of xanthone glycosides, such as 8-glucosyl-3-methoxy-1,7-dihydroxyxanthone [5]. CaCO₃ has been used for xanthone isolation. This stationary phase was carried out on various types of xanthenes, such as xanthenes hydroxylated, prenylated, and cyclized. The compound that was separated using CaCO₃ columns is α-mangostin in Figure 1, but this



method was not effective. The separation of α -mangostin shown in TLC has the same spot (1).

Figure 1. α -mangostin structure (1)

The best stationary phase for xanthone separation is using silica gel. Silica gel gives the best results compared to other stationary phases, but silica gel is not eco-friendly [7]. CaO as an absorbent research was developed several years ago [8]. CaO is a microparticle that has a high electronegativity. CaO and silica gel have the same pore size, but CaO is more eco-friendly than silica gel. According to Li et al. [6], xanthones were successfully isolated using a CaO column.

Therefore, in this work, we used CaO as a stationary phase to isolate a major compound in pericarp mangosteen. The analysis of the isolated compound was carried out using FTIR and NMR. Moreover, this research was followed by the antibacterial activity of an isolated compound.

MATERIALS AND METHODS

Materials

The materials used were dried pericarp of mangosteen was collected at Lingsar-Narmada, West Nusa Tenggara, Indonesia. The organic solvent used in this study were *N*-hexane, acetone, chloroform (Merck), ethyl acetate, methanol, and aquadest. Muller Hinton agar (MHA), sodium chloride (NaCl) 0,9% (b/v), and dimethyl sulfoxide (DMSO) used for antibacterial activity tested of isolated compound against *S. aureus* and *E. Coli*.

TLC was carried out using silica gel 60F₂₅₄ (Merck) and visualized under UV light (254 nm). VLC and gravity column chromatography (GCC) using CaO (Merck) and silica gel 60 (0063-0200 nm) by Merck 7734. Infrared (IR) spectra were recorded on a Shimadzu 8210PC. Nuclear Magnetic Resonance (NMR) was recorded at ¹H, ¹³C,

HSQC, and HMBC. This instrument operated at 500 MHz (¹H) and 125 MHz (¹³C) and used chloroform as a solvent.

Methods

Isolation and Characterization of Xanthones

The dry sample powder (1.79 kg pericarp of mangosteen) was extracted by the maceration method, following the procedure described by Ibrahim [6]. maceration using acetone for 3 to 24 hours. The acetone extract was separated from the powdered residue by filtration. The extract was concentrated by vacuum rotary evaporation until a thick extract of the sample was obtained.

Purification was performed using VLC and TLC [6]. The stationary phase used 100% CaO. The thick acetone extract (22 g) was mixed with impregnated silica gel 60 (0063-0200 nm) by Merck 7734. The column was eluted with a gradient of *n*-hexane and ethyl acetate and was done with an increasing polarity system. The results of VLC fractionation were monitored on TLC profiles and seen in UV light (254 nm). The fractions that gave similar TLC patterns or retention factors (R_f) were combined. The resulted in fractions of solution were evaporated and weighed. The selected fraction was further purified using GCC with silica gel 60 (0063-0200 nm) as the stationary phase. The purification method used the same treatment as the VLC. The purification result was expected to be a pure compound and was determined using IR and NMR spectroscopy.

Antibacterial Activity of Xanthones

The antibacterial activity was carried out by the modified agar disc diffusion method [7-9]. The bacteria used in this study were from the American Type Culture Collection (ATCC) *E. coli* ATCC 25922 (Gram negative) and *S. aureus* ATCC 25923 (Gram positive). The MHA was dissolved in aquadest and autoclaved at 121.1 °C for 60 minutes at a pressure of 2 atm.

The single colony of the testing bacterium was transferred into MHA medium in a saline sterile solution (NaCl 0,9%) and incubated for 24 hours at 37 °C. The positive control treatment used was ciprofloxacin, and DMSO as a negative control was also used as solvent. The testing procedure was carried out in three medium plates. Each plate contained thick acetone extract, the isolated compound, and control solvent and was incubated for 24 hours at 37 °C. The samples were prepared to

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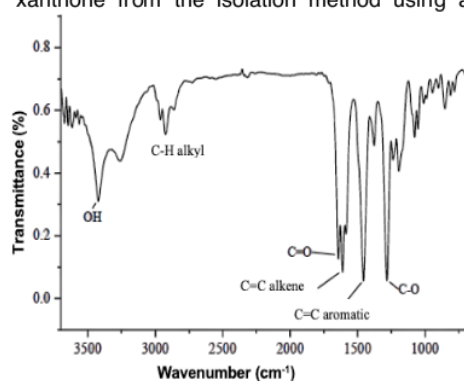
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a concentration of 50, 100, and 200 ppm in DMSO. Inhibition zones were measured and recorded.

RESULTS AND DISCUSSION

The maceration results yielded a thick acetone extract weighing 270.28 g (15.04%). Part of the extract (20 g) was fractionated by the VLC and GCC methods using eluent *n*-hexane: EtOAc of increasing polarity (9:1, 4:1, 7:3, 3:2, 1:1, 2:3, 3:7, and 1:4). The TLC profile result obtained a pure fraction of 5 mg of isolated compound. The isolated compound was verified by using the 2D-TLC system using different eluent *n*-hexane: EtOAc (8:2) and ethyl acetate: CH₃OH (7:3). The isolated xanthone from the isolation method using a



CaO column was less polar than using a silica column.

Figure 2. IR spectrum of (1)

The isolated compound was characterized using IR in the mid-infrared region, corresponding to wavenumbers of 4.000–650 cm⁻¹ (Figure 2). An IR spectrum shows the characteristic peaks of xanthones, such as hydroxyl (OH), carbonyl (C=O), C=C alkene and aromatic, and ester group (C-O-C). The band in the region of 1284 cm⁻¹ is assigned to the stretching and deformation of the ester group that is the identity of xanthone. The wide and strong peak at 3426 cm⁻¹ shows a hydroxyl chelate from the conjugated electron of the carbonyl that is shown at 1640 cm⁻¹. The sharp bond at 1614 and 1583 cm⁻¹ shows C=C alkene and C=C aromatic with a conjugated ring. This result is also supported by some spectra at 3060 cm⁻¹ and strong peaks at 1075 cm⁻¹ and 848 cm⁻¹ that show an aromatic ring. C-H aliphatic is identified at 2966, 2928, and 2858 cm⁻¹.

The ¹H-NMR spectroscopic data of 1 has similarities with the compounds isolated

[10] and shown in Table 2. The correlation between proton and carbon for hydroxy, prenyl, and group methoxy was supported by the HMBC correlations shown in figure 2. From HMBC correlations (Figure 2), the methoxy group and methylene prenyl (δH 3.45 ppm) group are in the same position as those in C-7. The HMBC spectrum also confirmed the hydroxy group in C-1 (δC 160.74 ppm). Two hydroxy groups cannot be observed in the spectrum, but the presence of the two hydroxy groups was seen by the presence of carbon signals C-3 (δC 161.78 ppm) and C-6 (δC 154.66 ppm), which is a typical shift for oxygenated carbon. It can also be indicated by C-3 and C-6, which is a quaternary carbon (Figure 3).

Two samples were tested for their antibacterial activity against *S. aureus* and *E. coli*. The result is shown in Table 1. The samples showed a significant effect on the growth of *S. aureus* and *E. coli* in an agar medium. This result was supported by the presence of carbonyl and hydroxyl groups in xanthones that break the lipid and amino acid.

Table 1. Inhibition of acetone extract and α-Mangostin against *S. aureus* and *E. coli*

Sample	Concentration (ppm)	Zone Inhibition (mm)	
		<i>S. aureus</i>	<i>E. coli</i>
Acetone extract	50	15.67	12
	100	16.67	13
	200	18	14
α-Mangostin	50	12.16	12
	100	13.33	13
	200	17	14
Negative control (DMSO)	100	-	-
Positive control (Ciprofloxacin)	100	28	37.3

The above results showed that acetone extract was more active in inhibiting the growth of *S. aureus*. This caused a synergic effect of the compounds in acetone extract. The α-Mangostin was resistant to bacteria (zone inhibition, 14 mm), especially in *S. aureus*. Antibacterial compounds were not easy to inhibit in bacteria like *E. coli* because there are three layers of a complex cell surface, such as peptidoglycan, lipoproteins, and polysaccharides, with 11-22% lipid. Based on the results, it seems that the antibacterial activity of xanthones in the pericarp of mangosteen might be worth investigating further.

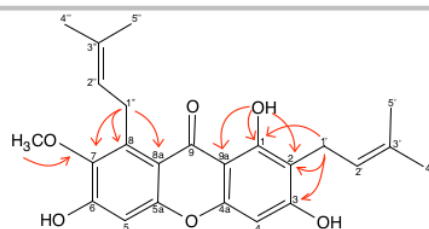


Figure 3. Selected key HMBC correlation of the α -mangostin (1)

Tabel 2. NMR spectroscopic data of isolated compound with literature values in CDCl_3

Position	Isolated Compound (ppm)		HMBC (1H \leftrightarrow 13C)	α -mangostin ^[11] (ppm)	
	δ_{H} (mult, J dalam Hz)	δ_{C}		δ_{H} (mult, J dalam Hz)	δ_{C}
1		160.74			161.60
2		108.55			111.00
3		161.78			163.10
4	6.29 (s)	93.45	C-2, C-3, C-4a, C-9a	6.41 (s)	93.00
4a		155.21			155.60
5	6.82 (s)	101.70	C-5a, C-6, C-7, C-8a	6.83 (s)	102.60
5a	-	155.93	-	-	155.10
6	-	154.66	-	-	157.50
7	-	142.66	-	-	144.40
8	-	137.17	-	-	138.00
8a	-	112.34	-	-	111.80
9	-	182.18	-	-	182.80
9a	-	103.77	-	-	103.50
1'	3.45 (d, 2H, $J = 7.25$)	21.59	C-1, C-2, C-3, C-2', C-3'	3.34 (d, 2H, $J = 7.3$)	21.90
2'	5.28 (m)	121.57	C-4', C-5'	5.28 (m)	123.40
3'	-	135.99	-	-	131.30
4'	1.84 (dd, 6H, $J = 1.3$)	18.37	C-3', C-5'	1.78 (s, 3H)	17.80
5'	1.77 (t, 3H)	25.98	C-2', C-3', C-4'	1.66 (s, 3H)	25.80
1''	4.09 (d, 2H, $J = 6.4$)	26.72	C-7, C-8, C-8a, C-2'', C-3''	4.13 (d, 2H, $J = 6.4$)	26.80
2''	5.28 (m)	123.28	C-4'', C-5''	5.28 (m)	124.70
3''	-	132.33	-	-	131.30
4''	1.84 (dd, 6H, $J = 1.3$)	18.07	C-3'', C-5''	1.83 (s, 3H)	18.20
5''	1.69 (d, 3H, $J = 1.55$)	26.01	C-2'', C-3'', C-5	1.66 (s, 3H)	25.90
1-OH	13.78 (s, OH)	-	C-1, C-2, C-9a	13.80 (s, OH)	-
3-OH	-	-	-	-	-
6-OH	-	-	-	-	-
7-OCH ₃	3.80	62.08	C-7	-	-

CONCLUSION

α -Mangostin was successfully isolated from pericarp mangosteen using VLC and CaO as stationary phases. The structure of α -Mangostin was identified conclusively by IR and extensive ^1H and ^{13}C NMR spectral analysis and comparison with literature data. The isolated compound has more active against *S. aureus* ATCC 25923 than *E. coli* ATCC 25922.

ACKNOWLEDGMENTS

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