



# Antibacterial Activity of Benzophenones Derived from the Ripe Fruit Extract of *Garcinia xanthochymus* against *Streptococcus mutans*

Patamaporn Pruksakorn <sup>a\*</sup>, Suwan Lertru <sup>b</sup>,  
Detmontree Wachisunthon <sup>a</sup>, Kamonwan Wanpen <sup>a</sup>,  
Parnuphan Panyajai <sup>c</sup> and Pornsri Prasertvaree <sup>a</sup>

<sup>a</sup> Department of Medical Sciences, Medicinal Plant Research Institute, Nonthaburi, 11000, Thailand.

<sup>b</sup> Department of Medical Sciences, National Institute of Health, Nonthaburi, 11000, Thailand.

<sup>c</sup> Department of Medical Sciences, Medical Life Sciences Institute, Nonthaburi, 11000, Thailand.

## Authors' contributions

This work was carried out in collaboration among all authors. Author PP designed the study, conducted the extraction, isolation, and identification of active substances, assessed brine shrimp toxicity, and wrote the first draft of the manuscript. Author SL evaluated antibacterial activity. Author

DW analyzed the molecular mass, while author KW assessed the optical rotation of active substances. Author PP calculated the LC<sub>50</sub> values, and author PP prepared the plant samples. All authors read and approved the final manuscript.

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## ABSTRACT

**Aims:** The objective of this study is to isolate antibacterial substances from the ripe fruit extract of *Garcinia xanthochymus* and to evaluate their toxicity through a brine shrimp lethality assay.

\*Corresponding author: Email: [patamaporn.p@dmsc.mail.go.th](mailto:patamaporn.p@dmsc.mail.go.th);

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**Study Design:** The research involved the collection of ripe fruits, followed by the isolation of active substances and subsequent biological activity assessments.

**Place and Duration of Study:** Ripe fruits of *G. xanthochymus* were collected in July 2022 from Ratchaburi Province, Thailand. The experiment work was conducted at the Department of Medical Sciences from July 2022 to September 2024.

**Methodology:** The isolation of active substances was conducted following the bioassay-guided separation principle. Biological activity evaluations were performed using the broth microdilution assay and the brine shrimp lethality assay.

**Results:** Benzophenones were isolated from the ethyl acetate extract of *G. xanthochymus* fruits, identified as the primary antibacterial agents. The isolated substances include guttiferone E (1a), xanthochymol (1b), isoxanthochymol (2a), and cycloxanthochymol (2b). These substances exhibited antibacterial activity against *Streptococcus mutans*, with minimum inhibitory concentrations (MICs) in the range of 6.25-12.5 µg/mL. Toxicity assessments indicated that guttiferone E (1a)/xanthochymol (1b) displayed brine shrimp toxicity, with a lethal concentration 50% (LC<sub>50</sub>) value of 3.67 µg/mL. Conversely, isoxanthochymol (2a)/cycloxanthochymol (2b) demonstrated a much lower toxicity profile, with LC<sub>50</sub> value exceeding 1,000 µg/mL.

**Conclusion:** The isolated benzophenones exhibited remarkable antibacterial activity against *S. mutans*, with MICs comparable to tetracycline. Additionally, the cyclized derivatives (2a and 2b) showed enhanced antibacterial activity and lower toxicity. These findings suggest the potential of isoxanthochymol (2a) and cycloxanthochymol (2b) for future antibacterial applications.

**Keywords:** *Garcinia xanthochymus*; antibacterial; *Streptococcus mutans*; benzophenones.

## 1. INTRODUCTION

*Garcinia xanthochymus* Hook.f. ex T. Anderson, commonly known as Egg tree, false mangosteen, and yellow mangosteen, is a tree belonging to the Clusiaceae (Guttifera) widely distributed in Southeast Asia, India, Nepal, Bhutan, Bangladesh, and China. The plant is a middling-sized tree, growing 8-10 meters tall. The leaves exhibit an elliptic or oblong to oblong-lanceolate shape, measuring 6-12 centimeters wide and 20-34 centimeters long, with shiny and thickly leathery. The petiole measures 1.5-2.5 centimeters long. The plant bears unisexual flowers on the same individual, clustered in the leafless axils, typically comprising 5-10 flowers per cluster. The flower features 5 petals and a

pedicel length of 1.8-3 centimeters. The fruit is green in color, broadly ovoid to spherical, with a smooth surface that ripens to a dark yellow color (Fig. 1). The interior of the fruit is juicy and contains yellow latex, with 3-5 seeds present [1-3]. This plant has been used in various traditional medicines for the treatment of diarrhea, dysentery, nausea, vomiting, and skin diseases [4-5]. There are many phytochemical constituents that can be found and extracted from *G. xanthochymus*, including xanthonones, benzophenones, flavonoids, isocoumarins, and depsidones [4]. Furthermore, this plant exhibits a variety of pharmacological activities such as antioxidant, anti-inflammatory, antidiabetic, and antimicrobial properties [4-6].



**Fig. 1.** The fruits of *G. xanthochymus*

The ripe fruits of *G. xanthochymus* are edible and exhibit a sweet and sour flavor profile. They have been used to make jams, vinegar, beverages, and other food products [7]. The fruits possess remarkable nutrition value and are rich in essential nutrients, including dietary fiber, carbohydrates, proteins, and fats. It also contains beneficial vitamins and minerals, such as carotenoids, vitamin C, potassium, calcium, magnesium, sodium, zinc, and iron [5-6]. It has been reported that the methanol extract of the lyophilized peel, the methanol of the lyophilized pulp, and the ethanol extract of the sun-dried rind of *G. xanthochymus* fruits exhibited significant inhibitory effects on  $\alpha$ -amylase,  $\alpha$ -glucosidase, and lipase. These findings suggest considerable antidiabetic potential of *G. xanthochymus* fruits [6]. Additionally, the fruit extracts demonstrated antibacterial activity against both gram-positive bacteria, including *Staphylococcus epidermidis*, *S. haemolyticus*, *S. mutans*, *S. pyrogens*, *Lactobacillus acidophilus*, *Cutibacterium acnes*, and *Atopobium vaginae*, as well as gram-negative bacteria, such as *Neisseria gonorrhoeae*, *Porphyromonas gingivalis*, *Salmonella typhimurium*, *Shigella flexneri*, and *Vibrio cholera* [5,8]. Given the fruit extracts exhibited remarkable antibacterial activity against cariogenic bacteria, such as *S. mutans*, *L. acidophilus*, *S. epidermidis*, and *P. gingivalis*, at MIC values of 25-200  $\mu$ g/mL [8]. The fruit extract of *G. xanthochymus* might have potential for the development of oral health care products. However, research on the antimicrobial substances present in this plant remains limited. In the present study, we aimed to identify the antibacterial substances present in the ripe fruits of *G. xanthochymus* and examined their antibacterial activity specifically against *S. mutans*. Furthermore, we conducted a brine shrimp toxicity assessment of the fruit extract and its active substances.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The ripe fruits of *G. xanthochymus* were collected from Ratchaburi Province, Thailand, in July 2022. The plant was authenticated by botanists at the Medicinal Plant Research Institute. The voucher specimen was deposited at the Department of Medical Sciences Herbarium, Nonthaburi, Thailand, with herbarium specimen number DMSC.: 5327.

### 2.2 Preparation of Extracts

Air-dried and grinded fruits of *G. xanthochymus* (25 g) were extracted with organic solvents (*n*-hexane (J.T. Baker, USA), chloroform (RCI Labscan, Thailand), ethyl acetate (J.T. Baker, USA), butanol (RCI Labscan, Thailand), acetone (J.T. Baker, USA), ethanol (RCI Labscan, Thailand), methanol (J.T. Baker, USA)) at room temperature (250 mL, twice). The extract was concentrated under reduced pressure using rotary evaporator (Hei-VAP Advantage, Heidolph, Germany) and freeze dryer (DC801, Yamato, Japan) to yield the fruit extracts.

### 2.3 Isolation of Antibacterial Substances

Air-dried and grinded fruits of *G. xanthochymus* (160 g) were extracted with ethyl acetate (1.6 L, twice) at room temperature. The extract was concentrated under reduced pressure to yield 13.7 g of dried extract. The extract was subjected to silica gel column chromatography and eluted with ethyl acetate: *n*-hexane to yield eight fractions (F1–F8). Two major fractions exhibited potent antibacterial activity, including F4 eluted at ethyl acetate: *n*-hexane at 15:85 to yield light yellow powder (Mixture 1, 3.2 g), and F6 eluted at ethyl acetate: *n*-hexane at 20:80 to yield pale yellow solid (1.7 g). F6 was washed with *n*-hexane to obtain 286 mg of colorless amorphous powder (Mixture 2). The chemical structures of active substances were determined using spectroscopic data.

**Mixture 1** – Guttiferone E (1a)/Xanthochymol (1b) light yellow amorphous powder:  $[\alpha]_D^{+20} +80.55^\circ$  (c 0.1, CH<sub>3</sub>OH); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  1.44 (1H, m, H-6), 2.06 (1H, m, H-7), 2.36 (1H, m, H-7), 7.00 (1H, m, H-12), 6.66 (1H, m, H-15), 7.00 (1H, m, H-16), 2.57 (1H, m, H-17), 2.75 (1H, m, H-17), 5.10 (1H, m, H-18), 1.80 (3H, s, H-20), 1.73 (3H, s, H-21), 1.15 (3H, s, H-22), 1.00 (3H, s, H-23) 1.96 (1H, m, H-24), 2.15 (1H, m, H-24), 4.91 (1H, t, J = 6.5 Hz, H-25), 1.60 (3H, s, H-27), 1.53 (3H, s, H-28), 1.63 (1H, m, H-29), 2.15 (1H, m, H-29), 2.66 (1H, m, H-30), 4.77 (2H, brs, H-32 1a), 4.38 (2H, brs, H-32 1b), 1.56 (3H, s, H-33), 1.45 (2H, m, H-34 1a), 1.69 (2G, m, H-34 1b), 4.85 (1H, m, H-35 1a), 1.88 (2H, m, H-35 1b), 1.66 (3H, s, H-37 1a), 4.63 (1H, brs, H-37 1b), 1.54 (3H, s, H-38 1a), 1.69 (3H, s, H-38 1b); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  198.0 (C-1), 115.9 (C-2), 193.8 (C-3), 69.7 (C-4), 49.6 (C-5), 46.8 (C-6), 42.6 (C-7), 57.8 (C-8), 209.2 (C-9), 194.2 (C-

10), 128.2 (C-11), 116.5 (C-12), 143.3 (C-13), 149.4 (C-14), 114.4 (C-15), 124.2 (C-16), 26.3 (C-17), 120.2 (C-18), 135.1 (C-19), 26.8 (C-20), 17.6 (C-21), 22.7 (C-22), 27.0 (C-23), 28.9 (C-24), 123.9 (C-25), 132.9 (C-26), 26.1 (C-27), 18.0 (C-28), 36.2 (C-29 1a), 36.5 (C-29 1b), 43.6 (C-30 1a), 43.3 (C-30 1b), 148.1 (C-31 1a), 146.0 (C-31 1b), 112.6 (C-32 1a), 113.2 (C-32 1b), 17.9 (C-33 1a), 18.1 (C-33 1b), 32.6 (C-34 1a), 31.9 (C-34 1b), 123.6 (C-35 1a), 35.5 (C-35 1b), 132.0 (C-36 1a), 147.4 (C-36 1b), 25.9 (C-37 1a), 109.6 (C-37 1b), 18.2 (C-38 1a), 22.6 (C-38 1b); ESI-MS m/z 603.4 [M + H<sup>+</sup>]

**Mixture 2** – Isoxanthochymol (2a)/Cycloxanthochymol (2b) colorless amorphous powder:  $[\alpha]_D^{25} +102.75^\circ$  (c 0.1, CH<sub>3</sub>OH); <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>)  $\delta$  1.47 (1H, m, H-6), 2.02 (1H, m, H-7), 2.11 (1H, m, H-7), 7.13 (1H, d, J = 2.1 Hz, H-12 2a), 7.16 (1H, d, J = 2.1 Hz, H-12 2b), 6.72 (1H, d, J = 8.3 Hz, H-15 2a), 6.68 (1H, d, J = 8.3 Hz, H-15 2b), 6.93 (1H, dd, J = 2.0, 8.3 Hz, H-16 2a), 6.88 (1H, dd, J = 2.0, 8.3 Hz, H-16 2b), 2.34 (1H, m, H-17), 2.48 (1H, m, H-17), 4.77 (1H, m, H-18), 1.50 (3H, s, H-20), 1.52 (3H, s, H-24), 1.05 (3H, s, H-22), 0.90 (3H, s, H-25 2a), 0.91 (3H, s, H-25 2b), 2.02 (1H, m, H-24), 2.58 (1H, m, H-24), 4.91 (1H, m, H-25), 1.65 (3H, s, H-27), 1.60 (3H, s, H-28), 1.02 (1H, m, H-29), 2.85 (1H, m, H-29), 1.37 (1H, m, H-30 2a), 1.23 (1H, m, H-30 2b), 1.18 (3H, s, H-32 2a), 1.17 (3H, s, H-32 2b), 0.83 (3H, s, H-33 2a), 0.78 (3H, s, H-33 2b), 29.0 (2H, m, H-34), 5.17 (1H, m, H-35 2a), 2.04 (1H, m, H-35 2b), 2.20 (1H, m, H-35 2b), 1.73 (3H, s, H-37 2a), 4.74 (2H, s, H-37 2b), 1.59 (3H, s, H-38 2a), 1.67 (3H, s, H-38 2b); <sup>13</sup>C NMR (125 MHz, DMSO-d<sub>6</sub>)  $\delta$  170.4 (C-1 2a), 170.6 (C-1 2b), 124.8 (C-2), 193.3 (C-3 2a), 193.5 (C-3 2b), 67.5 (C-4 2a), 67.6 (C-4 2b), 45.5 (C-5 2a), 45.7 (C-5 2b), 45.1 (C-6), 38.2 (C-7 2a), 37.9 (C-7 2b), 50.6 (C-8 2a), 51.0 (C-8 2b), 206.4 (C-9), 191.4 (C-10), 128.8 (C-11), 115.2 (C-12 2a), 114.8 (C-12 2b), 145.1 (C-13 2a), 145.3 (C-13 2b), 150.9 (C-14 2a), 151.0 (C-14 2b), 114.9 (C-15 2a), 114.7 (C-15 2b), 121.9 (C-16), 25.0 (C-17), 120.5 (C-18 2a), 120.4 (C-18 2b), 132.6 (C-19 2a), 132.7 (C-19 2b), 25.7 (C-20), 17.9 (C-21), 22.0 (C-22), 26.1 (C-23), 28.7 (C-24), 125.1 (C-25), 132.5 (C-26), 25.6 (C-27), 17.8 (C-28), 27.5 (C-29 2a), 27.6 (C-29 2b), 42.2 (C-30 2a), 41.1 (C-30 2b), 86.3 (C-31 2a), 86.7 (C-31 2b), 28.3 (C-32 2a), 28.1 (C-32 2b), 20.9 (C-33), 29.0 (C-34), 121.9 (C-35 2a), 34.8 (C-35 2b), 131.9 (C-36 2a), 144.6 (C-36 2b), 17.9 (C-37 2a), 22.0 (C-37 2b), 25.7 (C-38 2a), 110.8 (C-38 2b); ESI-MS m/z 603.4 [M + H<sup>+</sup>]

## 2.4 Assays for Biological Activity Studies

**Broth microdilution assay:** *Streptococcus mutans* ATCC 25175T DMST 18777 was obtained from the DMST culture collection (Department of Medical Sciences, Thailand) and cultured on sheep blood agar at 37 °C in a 5% CO<sub>2</sub> atmosphere for 24-48 hours. The stock suspension of *S. mutans* was prepared and adjusted for turbidity equivalent to a 1.0 McFarland standard. The suspensions were further diluted 1:10 in Brain Heart Infusion (BHI) medium for preparing the test inoculum. The tests were conducted in multiwell microdilution plates in which 50  $\mu$ L of test medium containing the test sample was 2X (two-fold) more concentrated than the final concentration. DMSO (Sigma-Aldrich, USA) was used as a negative control, and tetracycline (Sigma-Aldrich, USA) was used as a positive control. The microdilution plates were inoculated with 50  $\mu$ L of test inoculum in each well and incubated at 37°C in a 5% CO<sub>2</sub> atmosphere for 24 hours. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the test samples that completely inhibited microbial growth. Following the determination of MIC, 10  $\mu$ L aliquots were taken in triplicate from each well exhibiting negative growth and spread onto BHI agar plates. These plates were then incubated at 37°C in a 5% CO<sub>2</sub> atmosphere, and the bacterial colony growth was assessed after 48 hours of incubation. The minimum bactericidal concentration (MBC) was defined as the lowest concentration at which no colonies were observed on the agar plate.

**Brine shrimp lethality assay:** Brine shrimp eggs (*Artemia salina*; Sanders, USA) were hatched in artificial sea water prepared from salt (AquaRise, Thailand) 38 g in 1 L distilled water supplemented with 6 mg of yeast extract (Gibco, USA) at room temperature for 48 hours. The assay was carried out by using the brine shrimp larvae (15-30 organisms) in 1 mL of sea water treated in triplicate with the samples at different concentrations (10, 30, 100, 300, and 1,000  $\mu$ g/mL). DMSO was used as negative control, and doxorubicin (Sigma-Aldrich, USA) was used as positive control. After 24 hours, the brine shrimp were examined and determined the mean percentage mortality. The lethal concentration to half of the larvae (lethal concentration 50%, LC<sub>50</sub>) value was calculated using the software GraphPad Prism 6.

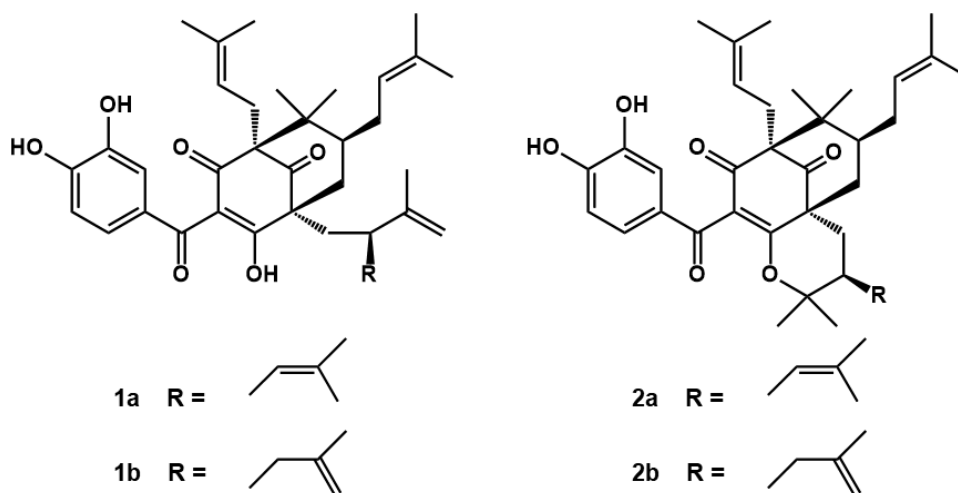
### 3. RESULTS AND DISCUSSION

Various studies have demonstrated the antimicrobial properties of the extracts from many parts of *G. xanthochymus* [5,8-10]. However, research on the antimicrobial substances present in this plant remains limited. In the present study, dried ripe fruits of *G. xanthochymus* were extracted using a range of organic solvents, and their antibacterial activity was evaluated against the cariogenic bacterium *S. mutans*. The results indicated that extracts obtained with hexane, chloroform, and ethyl

acetate exhibited strong antibacterial activity, with lower yields compared to those extracted with acetone, butanol, ethanol, and methanol (Table 1). These findings suggest that the antibacterial constituents may possess hydrophobic characteristics. Consequently, ethyl acetate was used to extract the dried ripe fruits of *G. xanthochymus* for the isolation of antibacterial substances. In addition, the fruit extracts showed bacteriostatic activity at MICs and exhibited bactericidal effects against *S. mutans* at concentrations equivalent to 8-fold the MIC (8XMIC).

**Table 1. Extraction yield and Antibacterial activity against *S. mutans* of the dried ripe fruit extracts of *G. xanthochymus***

Samples	% Yield (w/w)	MICs (µg/mL)	MBCs (µg/mL)
Hexane extract	6.16	25	200
Chloroform extract	7.04	25	200
Ethyl acetate extract	8.40	25	200
Acetone extract	12.34	50	400
Butanol extract	16.14	100	400
Ethanol extract	47.18	200	>400
Methanol extract	65.38	400	>400



**Fig. 2. Antibacterial substances isolated from *G. xanthochymus* fruits**

**Table 2. Biological activities of the ethyl acetate extract and active substances**

Test samples	Antibacterial activity against <i>S. mutans</i> (MICs, µg/mL)	Brine Shrimp Toxicity (LC <sub>50</sub> , µg/mL)
Ethyl acetate extract	25.0	9.92
Mixture 1: Guttiferone E (1a)/ Xanthochymol (1b)	12.5	3.67
Mixture 2: Isoxanthochymol (2a)/ Cycloxanthochymol (2b)	6.25	> 1,000
Garcinol*	50.0	ND
Tetracycline	12.5	ND
Doxorubicin	ND	13.82

\* Garcinol was purchased from MedChemExpress USA

To isolate the antibacterial substances, the ethyl acetate extract of *G. xanthochymus* fruits was fractionated using a silica gel column and eluted with mixtures of *n*-hexane and ethyl acetate. This process resulted in the isolation of two major active parts, 1 and 2. Structure determination by nuclear magnetic resonance (NMR) spectra and positive optical rotation indicated that 1 and 2 are mixtures of guttiferone E (1a)/xanthochymol (1b) and isoxanthochymol (2a)/cycloxanthochymol (2b), respectively (Fig. 2). These isolated benzophenones have been identified in various plant genera, such as *G. pyrifera* (fruits) [11], *G. ovalifolia* (leaves), *Clusia rosea* (leaves) [12], and *G. subelliptica* (pericarps) [13]. The benzophenones demonstrate a wide range of pharmacological activities, including anti-HIV [12], antibacterial [13], antiplasmodial [14], anti-inflammatory [15], antioxidant [16], and antitumor properties [17].

In this study, the isolated active substances were evaluated for antibacterial activity against *S. mutans* and toxicity on brine shrimp larvae. The isolated benzophenones, guttiferone E (1a)/xanthochymol (1b) and isoxanthochymol (2a)/cycloxanthochymol (2b), exhibited remarkable antibacterial activity, with MICs ranging from 6.25-12.5 µg/mL, nearly equal to the activity of the antibiotic tetracycline. Notably, the cyclized derivatives 2a and 2b displayed enhanced antibacterial activity against *S. mutans* compared to 1a and 1b. Additionally, these cyclized derivatives demonstrated lower toxicity in the brine shrimp lethality assay, with LC<sub>50</sub> value exceeding 1,000 µg/mL, while mixture 1 showed LC<sub>50</sub> value of 3.67 µg/mL. Furthermore, garcinol, the optical antipode of 1a, displayed reduced antibacterial activity relative to the isolated benzophenones, with MIC value of 50 µg/mL (Table 2). Xanthochymol (1b) has previously been reported to exhibit antibacterial activity against some methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA), with MICs values lower than those of garcinol [13]. This is consistent with our experimental findings. These results indicated that the configuration and specific atomic arrangement within the molecular structures are critical determinants of the antibacterial activity of benzophenones.

Guttiferone E (1a) and its double bond isomer, xanthochymol (1b) can be converted to isoxanthochymol (2a) and cycloxanthochymol (2b) through acid-catalyzed addition of the C1 enol to the terminal methylene in the side chain

[10-11]. The cyclized derivatives are relatively easy to synthesize from 1a and 1b, and they exhibited lower toxicity while remaining strong antibacterial activity. Therefore, isoxanthochymol (2a) and cycloxanthochymol (2b) represent promising candidates for the development of antibacterial health care products. The results of this study could support the antibacterial potential of the fruit extracts and isolated active substances derived from the ripe fruits of *G. xanthochymus* for use in oral health care products to protect dental caries.

#### 4. CONCLUSION

This study successfully isolated antibacterial substances from the ethyl acetate extract of *G. xanthochymus* fruits using silica gel column fractionation. The isolated mixtures, guttiferone E (1a)/xanthochymol (1b) and isoxanthochymol (2a)/cycloxanthochymol (2b), were characterized by NMR and optical rotation. Both mixtures demonstrated remarkable antibacterial activity against *S. mutans*, with MICs comparable to tetracycline, while the cyclized derivatives (2a and 2b) showed enhanced activity and lower toxicity. These findings highlight the potential of isoxanthochymol (2a) and cycloxanthochymol (2b) as promising candidates for future antibacterial applications.

#### DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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