Original Research Article

Antibacterial Activity of the Ripe Fruit Extract of *Garcinia xanthochymus* Against *Streptococcus mutans*

ABSTRACT

Aims: The objective of this study is to isolate antibacterial substances from the ripe fruit extract of *Garcinia xanthoxhymus* and to evaluate their toxicity through a brine shrimp lethality assay.
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. xanthoxhymus* were collected in July 2022from 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, identifiedas the primary antibacterial agents. The isolated substances includeguttiferone E (**1a**),xanthochymol (**1b**),isoxanthochymol (**2a**), andcycloxanthochymol (**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 thatguttiferone E (**1a**)/xanthochymol (**1b**)displayed brine shrimptoxicity, with a lethalconcentration 50% (LC_{50}) value of 3.67 µg/mL. Conversely, isoxanthochymol (**2a**)/cycloxanthochymol (**2b**)demonstrated a much lower toxicity profile, withLC_{50}value exceeding 1,000 µg/mL.
Conclusion: The isolated benzophenonesexhibited 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(Gutiferea) 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 anelliptic or oblong to oblong-lanceolate shape, measuring 6-12 centimeters wide and 20-34 centimeters long,withshiny andthickly leathery. The petiole measures 1.5-2.5 centimeters long.The plant bears unisexual flowers on the same individual, clustered in the leafless axils,

typicallycomprising5-10 flowersper cluster. The flower features 5 petals anda pedicel length of 1.8-3centimeters. The fruit is green in color, broadly ovoid to spherical,with a smooth surface that ripens to a dark yellow color (Figure 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, andskin diseases [4-5]. There are many phytochemical constituents that can be found and extracted from *G. xanthoxhymus*, including xanthones, 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. 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]. The fruit extractsdemonstrated antibacterial activity against both gram-positive bacteria,including *Staphylococcus epidermidis*, *S. haemolytica*, *Steptococcus mutans*, *S. pyrogens*, *Lactobacillus acidophillus, Cutibacterium acnes*, and *Atopobiumvaginae,* as well asgram-negative bacteria,such as *Neisseria gonorrhoeae*, *Porphyromonasgingivalis*, *Salmonella typhimurium*, *Shigella flexnerii*, and *Vibrio cholera* [5,7]. 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.* Additionally, we conducted a brine shrimp toxicity assessment of the fruit extract and its active substances.

2. MATERIAL 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: $[\alpha]_D$ +80.55° (*c* 0.1, CH_3OH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 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 1a0, 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);^{13}C NMR (125 MHz, $CDCl_3$) δ 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+]

Mixture **2** – Isoxanthochymol (**2a**)/Cycloxanthochymol (**2b**) colorless amorphous powder: $[\alpha]_D$+102.75° (*c* 0.1, CH_3OH); ^{1}H NMR (500 MHz, DMSO-d6) δ 1.47 (1H, m, H6), 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, H35 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); ^{13}C NMR (125 MHz, DMSO-d6) δ 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 (C15 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 (C31 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+]

2.4 Assays for Biological Activity Studies

2.4.1 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_2 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 multiwall microdilution plates in which 50 µL of test medium containing the test sample was 2X (twofold) more concentrated than the final concentration. DMSO (Sigma-Aldrich, USA) was used as negative control, and tetracycline (Sigma-Aldrich, USA) was used as positive control.The microdilution plates were inoculated with 50 µL of test inoculum in each well and incubated at 37°C in a 5% CO_2 atmosphere for 24 hours. The minimum inhibitory concentration (MIC) was defined as the lowest concentrations of the test samples that completely inhibited microbial growth.

2.4.2 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 distillated 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 µg/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_{50}) 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* [5,7-9]. 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 activitywas evaluated against the cariogenic bacterium *S. mutants*. 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. xanthoxhymus* for the isolation of antibacterial substances.

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)
Hexane extract	6.16	25
Chloroform extract	7.04	25
Ethyl acetate extract	8.40	25
Acetone extract	12.34	50
Butanol extract	16.14	100
Ethanol extract	47.18	200

Samples	% Yield (w/w)	MICs (µg/mL)
Methanol extract	65.38	400

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 bynuclear magnetic resonance (NMR) spectra and positive optical rotation indicated that **1** and **2** are mixtures of guttiferone E (**1a**)/xanthochymol (**1b**) andisoxanthochymol (**2a**)/cycloxanthochymol (**2b**), respectively (Figure 2).These isolated benzophenones have been identified in various plant genera,such as*G. pyrifera*(fruits)[10],*G. ovalifolis*(leaves), and*Clusia rosea*(leaves)[11]. The benzophenones demonstrate a wide range of pharmacological activities, including anti-HIV [11], anti-inflammatory [12], antioxidant [13], and antitumor properties [14].

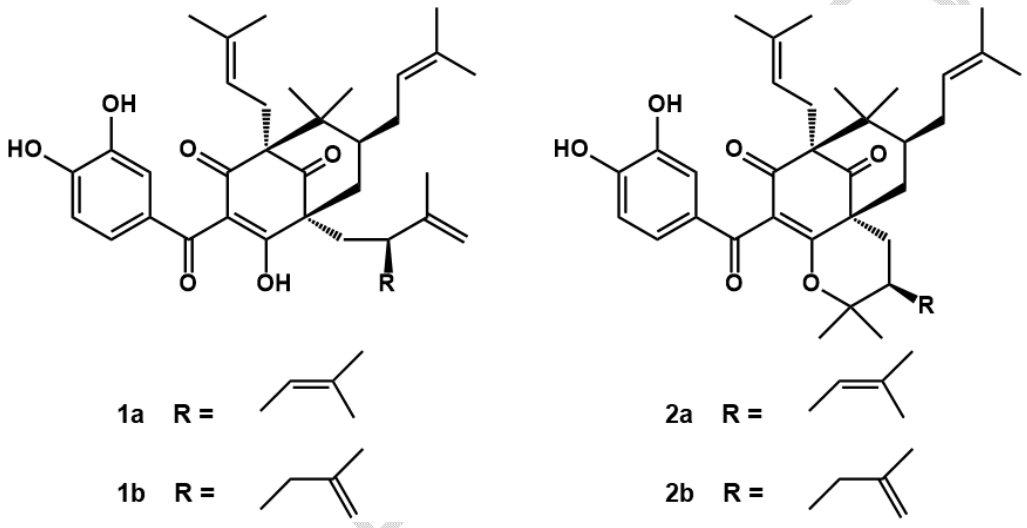

Fig. 2. Antibacterial substances isolated from G. xanthochymus fruits.

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 derivativesdemonstrated lower toxicity in the brine shrimp lethality assay, withLC_{50} value exceeding 1,000 µg/mL, whilemixture **1** showedLC_{50} 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). These findingsindicated that the configuration and specific arrangement of atoms within the molecular structuresarecritical determinantsofthe antibacterial activity of benzophenones.

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

Test samples	Antibacterial activity against *S. mutans* (MICs, µg/mL)	Brine Shrimp Toxicity (LC_{50}, µg/mL)
Ethyl acetate extract	25.0	9.92

Test samples	Antibacterial activity against *S. mutans* (MICs, µg/mL)	Brine Shrimp Toxicity (LC_{50}, µg/mL)
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.

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 synthesizefrom **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.

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.

REFERENCES

1. Gardner S, idisunthorn P, Chayamarit K, Utteridge T. Forest Trees of Southern Thailand. Bangkok: Kobfai Publishing Project.; 2015.
2. Hooker JD. Flora of British India. Kent: L. Reeve & Co. LTD.; 1875.
3. Xi-wen L, Jie L. Stevens P. 8. GARCINIA Linnaeus, Sp. P1. 1: 443. 1753.: Flora of China. 2007. Accessed 25 September 2024. Available: Garcinia in Flora of China @ efloras.org
4. Che Hassan NKN, Taher M, Susanti D. Phytochemical constituents and pharmacological properties of *Garcinia xanthochymus*- a review. Biomed Pharmacother. 2018;106:1378-89.
5. Murmu P, Kumar S, Patra JK, Singh NR, Rath SK. Ethnobotanical, nutritional, phytochemical and antimicrobial studies *ofGarcinia xanthochymus* fruit extracts. Br Biotechnol J. 2016;13(2):1-11.
6. Prakash J, Sallaram S, Martin A, Veeranna RP, Peddha MS. Phytochemical and functional characterization of different part of the *Garcinia xanthochymus* fruit. ACS Omega 2022;7(24):21172-82.
7. Pruksakorn P, Panyajai P, Jittaprasatsin C, SripichaiO,Sriboran S, Chaisomboonpan S. Antimicrobial activity and toxicity of the ethanolic extracts from *Garcinaixanthochymus*. J Thai Trad Alt Med. 2024;22(2):365-78. Thai.
8. Payamalle S, Murthy KN. Atomic force microscopic study for the antibacterial study of *Garcinia xanthochymus* Hook. F. Leaf extract. Int J Pharm Pham Sci. 2015;7(11):326-9.

9. Manohar SH, Naik PM, Patil LM, Karikatti SI, Murthy HN. Chemical composition *Garcinia xanthochymus* seeds, seed oil, and evaluation of its antimicrobial and antioxidant activity. J Herbs Spices Med P. 2014;20(2):148-55.
10. Roux D, Hadi HA, Thoret S, Guenard D, Thoison O, Pais M Sevenet T. Structure-activity relationship of polyisoprenyl benzophenones from *Garcinia pyrifera* on the tubulin/microtubult system. J Nat Prod. 2000;63(8):1070-6.
11. Gustafson KR, Blunt JW, Munro MHG, Fuller RW, McKee TC, Cardellina II JH, McMahon JB, Cragg GM, Boyd MR. The guttiferones, HIV-inhibitoty benzophenones from *Symphonia globulifera*, *Garcenialivingstonei*, *Garcinia ovalifolia* and *Clusia rosea*. Tetrahedron. 1992;48(46):10093-102.
12. Dzoyem JP, Lannang AM, Fouotsa H, Mbazoa CD, Nkengfack AE, Sewald N, Eloff JN. Anti-inflammatory activity of benzophenone and xanthone derivatives isolated from Garcinia (Clusiaceae) species. Phytochem Lett. 2015;14:153-8.
13. Baggett S, Protiva P, Mazzola EP, Yang H, Ressler ET, Basile MJ, Weinstein IB, Kennelly EJ. Bioactive benzophenones from *Garcinia xanthochymus* fruits. J
14. Riberio AB, de Melo MRS, de Melo Janqueira M, Rodrigues MGL, de Souza TO, Fernandes G, Santos MFC, Ambrosio SR, Bastos JK, Tavares DC. Efficacy and safety of guttiferone E in melanoma-bearing mice. Naunyn-Schmiedeberg's Arch Pharmacol. 2024;397:5265-74.