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Research Article

Isolation, Characterization and some Biological Activities of a Xanthone from *Garcinia Mangostana*

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***Abstract*— Chemical and biological studies were carried on the stem bark of *Garcinia mangostana*. A xanthone was isolated from the chloroform extract. The structure of this compound was established by spectral methods (IR, UV,^{1}H-NMR,^{13}C-NMR, HMQC,HMBC and MS). The crude extractives as well as the isolated compound were evaluated for their antibacterial and larvicidal potential. The antibacterial activity test against four standard microbes, *Staphylococcus aureus* (ATCC25923- G +ve), *Escherichia coli* (ATCC25922- G –ve), *Pseudomonas aeruginosa* (ATCC27853- G-ve) and *Proteus vulgaris* (NCTC8196). The crude chloroform and ethyl acetate extracts of *Garcinia mangostana* gave moderate inhibition against *S. aureus* only. The pure compound (I) showed moderate activity against *S. aureus*. The hexane, chloroform and ethylacetate crude extracts of *Garcinia mangostana* were screened for larvicidal activity against *Aedes aegypti*. The crude chloroform was very active, the crude hexane was moderately. The crude ethyl acetate and compound (I) devoid of activity.**

***Index Terms* — *Garcinia mangostana*, Isolation, Characterization, Xanthones and Biological activities.**

I. INTRODUCTION

Garcinia is a plant of the family *Glusiaceae* native to Asia, Australia, tropical and southern Africa and Polynesia.The genus *Garcinia* has more than fifty species [1]. The mangosteen is probably the most highly praised tropical fruit. The fruit rind is used to tan leather and to dye black. Both the rind and the bark have several applications in traditional medicine. The dark red wood is used in carpentry and to make rice pounders. The fruit rind is ground and used in the treatment of diarrhea and dysentery, it is also used for skin diseases [2].

Xanthones are secondary metabolites commonly occurring in a few higher plant families, fungi and lichen. Their high taxonomic value in such families and their pharmacological properties has roused great interest [3]. In 1977 Hostettmann and Wagne described one of the extensive groups of natural xanthones- xanthone glycosides [4]. The sharp growth of interest in natural xanthones in recent years can be readily accounted for by the fact that the first studies in the field of pharmacological properties of xanthone derivatives indicated promising activity [5].

Xanthones are indeed rare antioxidants [6] that are only found in few fruits like mangosteen. Xanthones have strong cancer fighting components, it has been found that a class of xanthones called- α -mangostin can be used to halt the development of tumors [7]. Another type called garcicone E can prevent cancer in essential body organs such as liver, lung, and colon. Xanthones are polyphenolic compounds that commonly occur in herbs such as *Swertia davidi* France (*Gentianceae*), which has been used in the treatment of inflammation, allergy or hepatitis [8]. There are only a few studies on the toxicity of xanthones[9,10]. Although in some studies xanthones were found to be cytotoxic in tumor cell lines, they are generally considered to have low toxicity to normal cells and tissues**.**

The objectives of this study are

-Extraction of an active xanthone from the medicinally important species *Garcinia mangostana*.

-Elucidation of the structure of the isolated xanthone via spectroscopic tools: UV, IR, ^{1}H-NMR, ^{13}C-NMR, ^{1}H-^{1}H cosy NMR, HMQC and HMBC.

-`Screening of the crude extracts and the isolated xanthone for their antibacterial and antilarvicidal potential.

II. MATERIALS AND METHODS

Plant Material

Garcinia mangostana was collected from Universiti Putra Malaysia (UPM) Selangor and kindly authenticated by Botany Department- University Putra Malaysia.

Methods

Extraction and Isolation of phenolics from Garcinia mangostana

1.7Kg of powdered air dried stem bark of *Garcinia mangostana* was extracted with distilled hexane for forty eight hours at room temperature. The hexane extract was filtered and the residue was then re-extracted with a fresh portion of distilled hexane. The extract was then concentrated under reduced pressure. This procedure was repeated by replacing hexane with chloroform and ethyl acetate successively. Yields were found to be 28.08g (hexane extract), 79.25g (chloroform extract) and 45.03g (ethyl acetate extract).

Isolation of Compound (I)

The chloroform extract of *Garcinia mangostana* stem bark gave compound (I).The chloroform extract was fractionated by mini CC using hexane, hexane/ chloroform, to afford 11 fractions. Fraction 10 (90%chloroform: 10%hexane) was a yellow amorphous powder. It was recrystallized using chloroform to give compound (I) (57mg); m.p. 179-181C^{o}.

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Antibacterial Activity

Preparation of bacterial suspension

One ml aliquots of 24 hours broth cultures of tested organisms (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*) were aseptically distributed onto nutrient agar slopes and incubated at 37^{o}C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline, to produce a suspension containing 108-109 colony forming units per ml. The suspension was stored in the refrigerator at 4^{o}C till used. Serial dilutions of the stock suspension were made in sterile normal saline, and 0.02ml volumes of the appropriate dilution were transferred onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37^{o}C for 24 hours.

Testing for antibacterial activity

The cup-plate agar diffusion method [11] was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. 1ml of the standardized bacterial stock suspension was mixed with 100ml of molten sterile nutrient agar which was maintained at 45C^{o}. 20ml aliquots of the incubated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set and in each of these plates 4 cups (10mm in diameter) were cut using sterile cork borer (No.4) and agar discs were removed. Alternate cups were filled with 0.1ml sample of each of the extracts using adjustable pipette, and allowed to diffuse at room temperature for two hours.

The plates were then incubated in the upright position 37^{o}C for 18 hours. These tests were carried out for crude extracts and pure compound against each of the tested organisms. After incubation the diameter of the resultant growth inhibition zones were measured.

Larvicidal Assay

The assay were carried out to investigate the toxicity of crude extracts and pure compound against the larvae of mosquito of *Aedes aegipti* (from institute of medicinal research Malaysia) using the WHO standard procedure with slight modifications [12]. A stock solution was prepared at a concentration of 1000ppm (mg/l) by dissolving 100mg of sample in 10ml of absolute ethanol. For the initial test, three test solutions at 50ppm, 100ppm and 150ppm were prepared by transferring 0.25ml, 0.5ml and 0.75ml of stock solution, respectively into three drinking glasses which were previously filled with 25ml chlorine- free tap water. 10 late third in star mosquito larvae were introduced into each glass by using a droper. The test solution was made up to 50ml with chlorine- free tap water and a small amount of larval food (roasted chicken liver) was added. A control was prepared in the same way by using 0.75ml of absolute ethanol instead of the stock solution. Mortality counts were made after 24 hours. A series of at least five concentrations was then made within the range in triplicates. The mortality data were analyzed using the Probit Analysis Programme to obtain LC50 and LC90 values.

III. RESULTS AND DISCUSSION

Spectral data of compound (I)

IR νmax (Cm^{-1} ,KBr disc): 3410, 2962, 2916, 1640, 1451, 1377, 1050, 808 and 781.

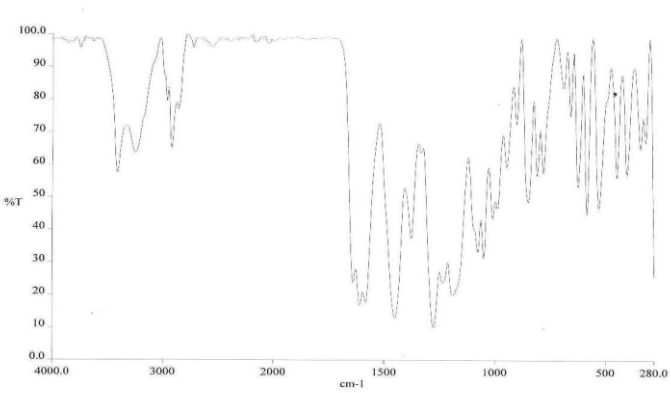

Figure (1)

UV λ max (MeOH nm): 215, 245, 318.

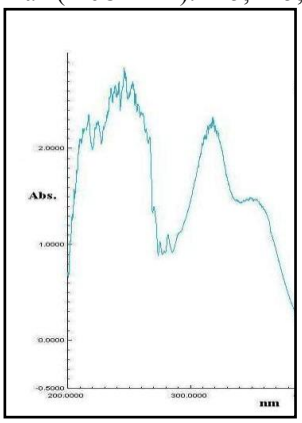

Figure (2)

^{1}HNMR (400MHz, CDCl3):

δ13.78, δ6.81, δ6.28, δ5.26, δ4.08, δ3.80, δ3.44, δ1.84, δ1.83, δ1.77,δ1.69.

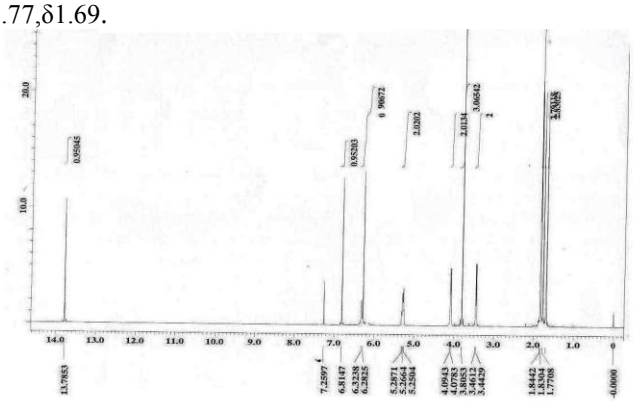

Figure (3)

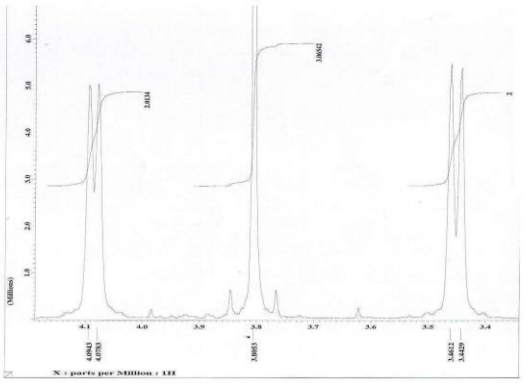

Figure (4)

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^{1}H-^{1}H COSY spectra of compound (I):

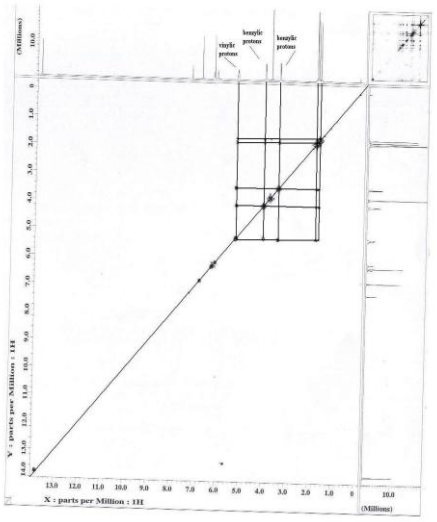

Figure (5)

^{13}C NMR, δ(100MHz, CDCl3):

δ181.9 , δ161.58 , δ160.5 , δ155.7 , δ155.02 , δ154.4 , δ142.4 , δ137 ,
δ135.8 , δ132.1, δ123.1, δ121.4 , δ112.1 , δ108.4 , δ103.5, δ101.5,
δ93.3 , δ62.0 , δ26.5 , δ25.83 , δ25.81 , δ21.4 , δ18.2 , δ17.9.

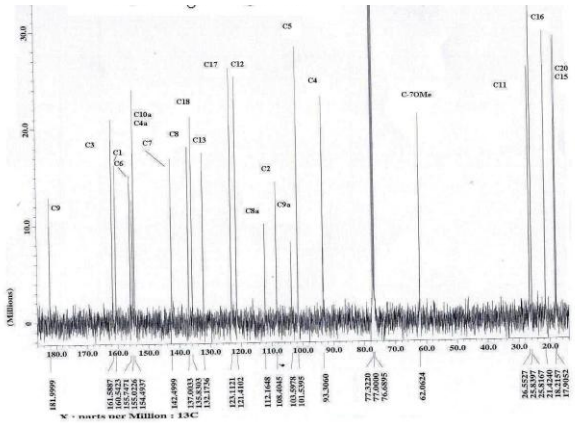

Figure (6)

HMQC spectrum of compound (I):

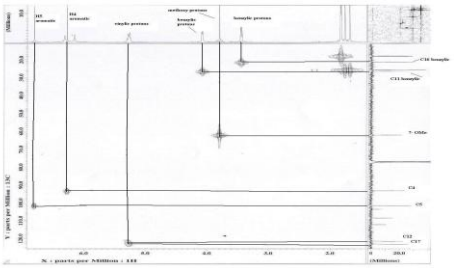

Figure (7)

HMBC of compound (I):

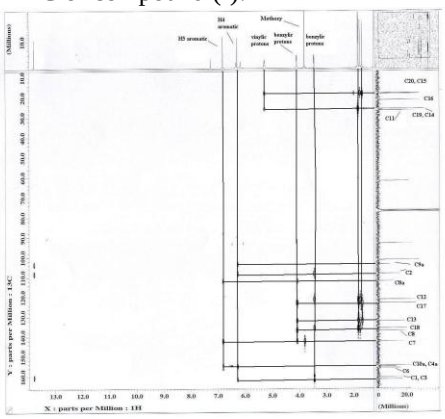

Figure (8)

The mass spectrum of compound (I):

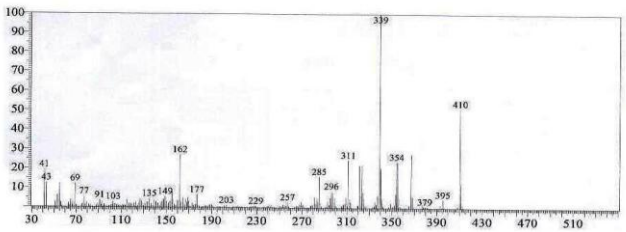

Figure (9)

Table (1). Antibacterial activity of crude extracts and pure compound for the studied plant

Sample	Target Microbe			
	S. aureus	*E. coli*	*P.aeruginosa*	*P. vulgaris*
Chloroform Extract	11	-	-	-
Ethyl acetate Extract	13	-	-	-
Pure compound (I)	12	-	-	-

Concentration used = 100 g/ml
- mm = no inhibition
1-9 mm = weak inhibition
10-14mm = medium inhibition
15-19mm = strong inhibition

Table (2). Larvicidal activity of crude extracts and compound (I) against the larvae *Aedes aegypti*

Sample	LC_{50} µg/ ml	LC_{90} µg/ml
Hexane Extract	149.7	475.8
Chloroform extract	20.8	112.1
Ethyl acetate Extract	N.A.	N.A.
Pure compound (I)	N.A.	N.A.

N.A. =Not active
LC50 ≤ 50µ*g /ml* : very active
LC50 between 50-100µ*g /ml* : active
LC50 between 100-150µ*g /ml* : moderately active
LC50 between 150-250µ*g /ml* : weakly active

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In the IR spectrum compound I gave ν (KBr) 720, 808(C-H, Ar. bending), 1050 (C-O), 1377, 1451 (C=C, Ar.), 1640 (C=O), 2962, 2916(C-H), 3410(broad OH) (Fig. 1).

The UV spectrum of compound I gave λ_{max} (MeOH): 245, 318nm corresponding to two benzoyl chromophores characteristic of xanthons(Fig. 2). The ^{1}H NMR spectrum of compound (I) gave four signals at δ 1.83 (s, 3H), δ 1.84(s, 3H), δ 1.77(s,3H) and δ 1.69(s, 3H) due to four methyl groups. The signals at δ 3.44(d, 2H) and 4.09(d, 2H) were assigned to two benzylic methylene groups. The resonance atδ 5.26(t, 2H) corresponds to vinylic protons. The above signals suggest the presence of two isoprenyl moieties. The signal at δ 3.8(s, 3H) is characteristic of a methoxyl function, while the resonances at δ 6.28 and 6.8ppm are due to two aromatic protons(Figs. 3and 4). The^{1}H-^{1}H COSY spectrum demonstrated long range coupling between vinylic protons at δ 5.26 and methyl groups at δ 1.83,δ 1.84, δ 1.77 and δ 1.69ppm. The correlation between the vinylic protons at δ 5.26 and the benzylic methylene protons atδ 3.44 and δ 4.09 is citing evidence for two isoprenyl moieties. Also the^{1}H-^{1}H COSY experiment indicated long range coupling between a vinylic proton at δ 5.26 and a methoxy moiety at δ 3.8 (Fig. 5). The ^{13}C spectrum was characteristic of a C24 – skeleton (Fig.6).

The HMQC spectrum (Fig.7). showed correlations between the vinylic proton signal at δ 5.26 and the carbon signals at δ 121 and δ 123.1; the aromatic proton at δ 6.2 and the carbon signal at δ 93.3; the aromatic proton at δ 6.8 and the carbon signal at δ 101; the benzylic protons signal at δ 3.4 and the carbon signal at δ 21.4,;the benzylic protons signal at δ 4.9 and the carbon signal at δ 26; the methoxy protons at δ 3.8 and the carbon signal at δ 62 The HMBC spectrum showed correlation between the methylene signal of the isoprenyl moiety at δ 4.09 and the carbon signals at δ 142.4 (C-7), δ 121.4, δ 132.1, δ 112 (C-8a) andδ 137(C-8). Hence one of the isoprenyl groups was cited at C-8. Also it revealed correlation between the methylene resonance at δ 3.44 and the carbon signals at δ 161.5(C-3), δ 160.5(C-1), δ 135.8, δ 123, δ 25.7, δ 18.2 and δ 108.4 (C-2). This is providing evidence for a C-2 isoprenyl moiety .It also revealed correlation between the methoxyl protons (δ 3.8) and the resonance at δ 142.4(C-7) (Fig. 8). The mass spectrum of compound (I) gave m/z 410 for the molecular ion (M+) (Fig. 9).

The above cumulative data limits structure of compound I to the following structure (Fig. 10):

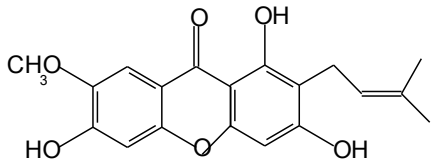

Figure (10)

The antibacterial activity of plant extracts and pure compound was investigated against four standard microbes, *Staphylococcus aureus* (ATCC25923- G +ve), *Escherichia coli* (ATCC25922- G –ve), *Pseudomonas aeruginosa* (ATCC27853- G-ve) and *Proteus vulgaris* (NCTC8196). The result showed that the crude chloroform and ethyl acetate extracts of *Garcinia mangostana* gave moderate inhibition against *S. aureus* only. The pure compound (I) showed moderate activity against *S. aureus* (**Table 1**).

The larvicidal activity of crude extracts and pure compounds was investigated against the larvae of *Aedes aegypti* using the WHO (1981) standard method with slight modifications. The results showed that the crude chloroform extract was very active, while the crude hexane extract was moderately active. The crude ethyl acetate extract and compound (I) devoid of activity (**Table 2**).

IV CONCLUSIONS

Extraction and purifying of the stem bark of *Garcinia mangostana* gave compound (I). The antibacterial activity test against four standard microbes, *Staphylococcus aureus* (ATCC25923- G +ve), *Escherichia coli* (ATCC25922- G –ve), *Pseudomonas aeruginosa* (ATCC27853- G-ve) and *Proteus vulgaris* (NCTC8196) were carried out on the crude extracts and the pure compounds. The crude chloroform and ethyl acetate extracts of *Garcinia mangostana* gave moderate inhibition against *S. aureus* only. The pure compound (I) showed moderate activity against *S. aureus*. The hexane, chloroform and ethylacetate crude extracts of *Garcinia mangostana* were screened for larvicidal activity against *Aedes aegypti*. The crude chloroform was very active, the crude hexane was moderately. The crude ethyl acetate and compound (I) devoid of activity.

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compounds anthraquinobenzophenones from *Cratoxylum sumatranum*, *J. Nat. Prod*., 65, 299-305.

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