Xanthones Isolated from the Pericarp of Mangosteen Inhibit Neurotransmitter Receptors Expressed in *Xenopus* Oocytes

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Objective: *This study aimed to evaluate inhibitory effects of 7 xanthones and 3 extracts obtained from the pericarp of mangosteen on serotonin (5-HT), N-methyl-D-aspartate (NMDA) and glycine receptors expressed in Xenopus oocytes.*
Material and Method: *Xenopus oocytes were injected with RNA of either 5-HT, NMDA or glycine receptor and inhibitory effects of the xanthones and extracts were investigated using the two-electrode voltage clamp technique.*
Results: *Xanthones from the pericarp of mangosteen affected 5-HT, NMDA and glycine receptor functions with different degree of inhibition. Alpha-mangostin, garcinone-D and 9-hydroxycalaba xanthone inhibited 5-HT-induced currents by more than 80%. Gamma-mangostin and garcinone-E did by more than 50%, but not more than 80%. Garcinone-C and garcinone-D inhibited glutamate-induced currents by more than 80%. The alcohol extract did by more than 50%, but not more than 80%. Alpha-mangostin, garcinone-C, garcinone-D, non-tannin extract and the alcohol extract inhibited glycine-induced currents between 50-70%, neither compounds inhibited the currents up to 80%.*
Conclusion: *These results suggest that each xanthone derivatives has different selectivity to different types of neurotransmitter receptors.*

Keywords: *Xanthones, Mangosteen, Two-electrode voltage clamp technique, 5-HT, NMDA and glycine receptors*

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The mangosteen (*Garcinia mangostana* L.), sometimes called the "Queen of Fruits", is a tropical evergreen tree distributed in Thailand and other Southeast Asian countries. The fruits are used in traditional medicine to treat abdominal pain, diarrhea, dysentery, infected wound and chronic ulcer[1]. The pericarp of mangosteen is a rich source of various phytochemicals including tannins and more than 40 xanthones with α-, β- and γ-mangostins (Fig. 1) as the dominating ones[2]. Recent studies have revealed that xanthones exhibit a variety of biological activities such as antituberculosis[3], anticancer[4-7], antioxidant[8], antihypertension[9], hypoglycemia[10], anti-platelet aggregation[11] and anti-seizure[12].

Xanthones are lipid soluble compounds, so that it is possible to enter the brain. They are claimed to prevent deterioration of brain diseases, including Parkinson's, Alzheimer's and other dementia, through their antioxidant activities. Studying in animals demonstrated sedative effects of xanthones on the central nervous system characterised by ptosis, sedation, decreased spontaneous motor activity, loss of muscle tone and potentiation of pentobarbitone

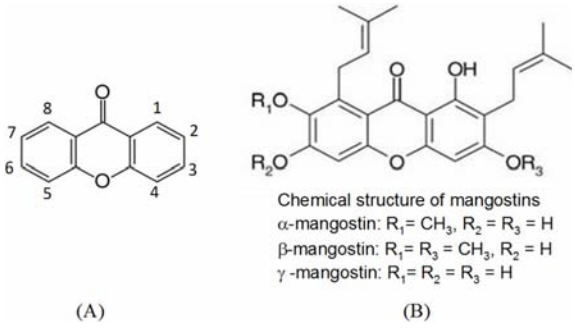

Fig. 1 Structures of xanthone (A) and major prenylated xanthones from the *G. mangostana* (B).

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sleeping time[13]. However, the mechanism underlying the sedative effects of the xanthones have not been clarified. Therefore, the aim of this study was to evaluate inhibitory effects of xanthones from the pericarp of mangosteen on excitatory function of neurotransmitter receptors including serotonin (5-HT), N-methyl-D-aspartate (NMDA) and glycine receptors.

Material and Method

Mangosteen extract preparation

Mangosteen fruit (*G. mangostana*) was collected from Kombang District, Chantaburi Province, Thailand. A voucher specimen (Ms Porntip Wongnapa No. 003) was deposited at the Faculty of Science, Srinakharinwirot University. The dried and pulverized pericarp of *G. mangostana* was thoroughly extracted with ethyl acetate at 50°C, the extract was then filtered and collected. The marc residue was further extracted with 95% ethylalcohol at 60°C. Each extract after filtration was evaporated to dryness under reduced pressure to give a non-tannin extract as a yellowish solid (MGT-8) and a tannin extract as a dark brown solid (MGT-9), respectively. In a likewise manner, the pericarp was extracted with 95% ethyl alcohol at 60°C to give an alcohol extract as a brown solid (MGT-10). Seven xanthones (MGT-1 to MGT-7) were further isolated and purified from the non-tannin extract as described previously (Fig. 2, 3)[2,3].

Oocyte injection

Defolliculated stage V-VI oocytes were prepared from *Xenopus laevis* (Xenopus Express, Cape, South Africa) as described previously[14]. Briefly, *Xenopus laevis* were anesthetized in ice-water, and a lobe of the ovary was dissected and placed in sterile modified Barth's solution (MBS: 88 mM NaCl, 1 mM KCl, 0.41 mM $CaCl_2$, 0.33 mM Ca $(NO_3)_2$, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 7.5 mM Tris (hydroxymethyl) aminomethane, pH 7.6). Oocytes were then isolated manually and defolliculated by incubation in 2 mg/ml collagenase (type IA; Sigma, St. Louis, MO, USA) at 19°C for 1 h in calcium-free MBS solution. To examine 5-HT or glycine receptor function, total RNA was prepared from whole brain or spinal cord of adult male rats using the Trizol reagent (Gibco-BRL), and oocytes were injected with 46 nl of the total RNA (5 mg/ml). To examine NMDA receptor function, cRNA was prepared from DNA clones of NR1a and NR2B kindly provided by Dr. K. Igarashi (Chiba University) and oocytes were injected with 27.6 nl of NR1a/NR2B RNA mixture (0.5 mg/ml). After injection, oocytes were incubated in MBS containing 2.5 units/ml penicillin and 2.5 µg/ml streptomycin at 19°C.

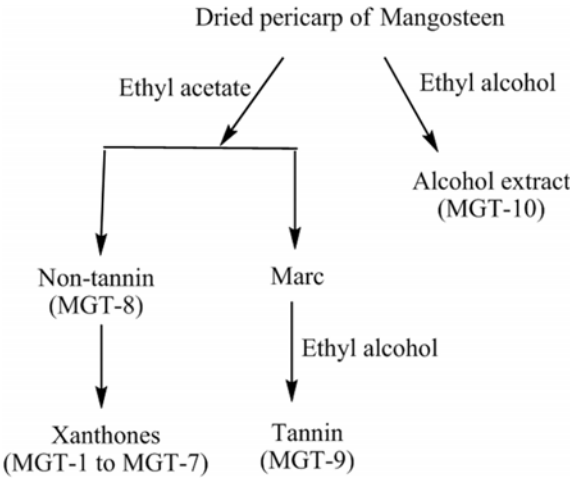


Fig. 2 Mangosteen extract preparation.

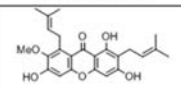
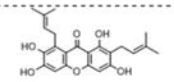
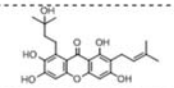
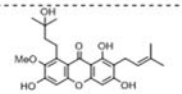
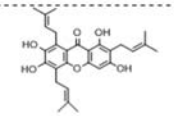
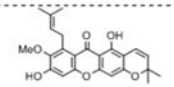
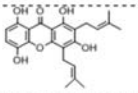
Code	Name	Chemical structure	Formula
MGT-1	α-Mangostin		$C_{24}H_{26}O_6$
MGT-2	γ-Mangostin		$C_{23}H_{24}O_6$
MGT-3	Garcinone-C		$C_{23}H_{26}O_7$
MGT-4	Garcinone-D		$C_{24}H_{28}O_7$
MGT-5	Garcinone-E		$C_{28}H_{32}O_6$
MGT-6	9-Hydroxycalabaxanthone		$C_{24}H_{24}O_6$
MGT-7	Gartanin		$C_{23}H_{24}O_6$
MGT-8	Non-tannin extract	-	-
MGT-9	Tannin extract	-	-
MGT-10	Alcohol extract	-	-

Fig. 3 Code, name and formula of the test compounds.

Electrophysiological recording

Responses to either 5-HT, NMDA or glycine

were recorded using a two electrodes voltage-clamp amplifier (Gene Clamp 500B; Axon Instrument, Foster City, CA, USA) at a holding potential of -60 mV unless noted otherwise. Electrodes were filled with 3 M KCl and had resistances of 0.5-5 MΩ. Oocytes were positioned in a 50-µl chamber and continuously perfused with MBS solution at 1 ml/min at room temperature. We investigated the effect of xanthones (MGT-1 to MGT-7) and the extracts (MGT-8 to MGT-10) by pretreating oocytes with the MGTs 1 min before coapplication with either 5-HT, NMDA or glycine. The drugs were applied until a plateau or peak of the response was observed. Data were recorded and digitized for analysis (MacLab/200 interface; AD Instruments, Castle Hill, NSW, Australia).

Statistical analysis

Most data was presented as percentages of control responses and expressed as the mean ± SD.

Results

Inhibitory effect of xanthones and the extracts on 5-HT receptor function

To examine inhibitory effect of MGT-1 to MGT-10 on 5-HT receptors, 0.1 µM 5-HT was applied to an oocyte in the presence or absence of MGT (20 µg/ml). The MGT-1 to MGT-10 inhibited the 5-HT receptor function by 96.8%, 67.3%, 26.8%, 88.7%, 79.1%, 87.0%, 0.0%, 4.5%, 7.5% and 24%, respectively (Fig. 4).

Inhibitory effect of xanthones and the extracts on NMDA receptor function

To examine inhibitory effect of MGT-1 to MGT-10 on NMDA receptors, 10 µM glutamate plus 10 µM glycine was applied to an oocyte in the presence or absence of MGT (20 µg/ml). The MGT-1 to MGT-10 inhibited the NMDA receptor function by 38.7%, 46.5%, 84.7%, 80.6%, 19.3%, 11.9%, 8.9%, 47.9%, 19.4% and 58.0%, respectively (Fig. 5).

Inhibitory effect of xanthones and the extracts on glycine receptor function

To examine inhibitory effect of MGT-1 to MGT-10 on glycine receptors, 300 µM glycine was applied to an oocyte in the presence or absence of MGT (20 µg/ml). The MGT-1 to MGT-10 inhibited the glycine receptor function by 59.3%, 70.2%, 62.0%, 46.2%, 36.1%, 1.8%, 29.1%, 49.7%, 13.2% and 60.1%, respectively (Fig. 6).

Discussion

The present study has demonstrated that xanthones (MGT-1 to MGT-7) and extracts (MGT-8 to

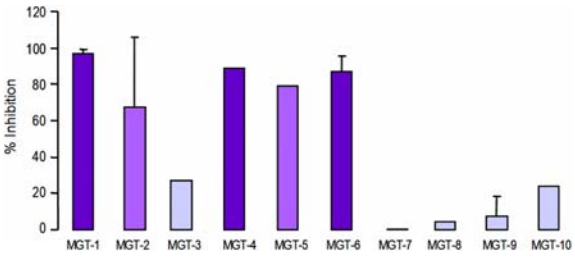


Fig. 4 Inhibitory effect of xanthones and the extracts on 5-HT receptors. Oocytes injected with total rat brain RNAs were applied with 0.1 µM 5-HT in the absence (control) or presence of the test compounds (MGT-1 to MGT-10) at 20 µg/ml. Data are expressed as the mean ± SD of percentages of control response from 1-3 oocytes.

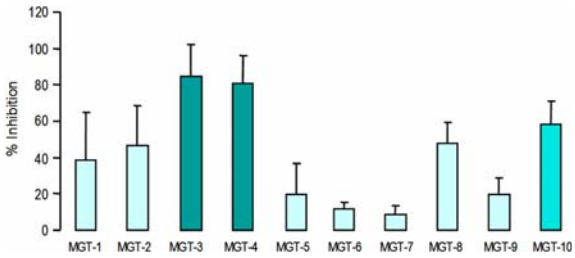


Fig. 5 Inhibitory effect of xanthones and the extracts on NMDA receptors. Oocytes injected with NR1a and NR2B cRNAs were applied with 10 µM glutamate plus 10 µM glycine in the absence (control) or presence of the test compounds (MGT-1 to MGT-10) at 20 µg/ml. Data are expressed as the mean ± SD of percentages of control response from 3-9 oocytes.

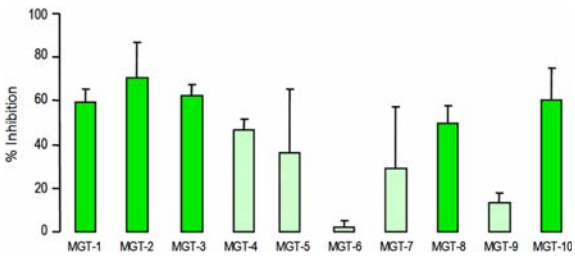


Fig. 6 Inhibitory effect of xanthones and the extracts on glycine receptors. Oocytes injected with total rat spinal cord RNAs were applied with 300 µM glycine in the absence (control) or presence of the test compounds (MGT-1 to MGT-10) at 20 µg/ml. Data are expressed as the mean ± SD of percentages of control response from 3-13 oocytes.

MGT-10) from the pericarp of the mangosteen can inhibit 5-HT, NMDA and glycine receptor functions with different degree at the same concentration. From these results, the inhibitory activity of the MGTs on function of each receptor was classified as high, moderate and low potency if the degree of inhibition is 80-100%, 50-79% and less than 50%, respectively. MGT-1, -4 and -6 inhibited 5-HT receptor with high potency, whereas MGT-2 and -5 did with moderate potency. Many anti-anxiety drugs act by inhibiting serotonin function[15,16]. So, MGT-1, -4 and -6 will have the potential to be agents for the treatment of anxiety. The potent NMDA inhibition by MGT-3 and -4 suggests that these MGTs might be useful in the treatment of Alzheimer's and Parkinson's diseases[17]. The inhibitory activity of MGT-1, -2, -3, -8 and -9 on glycine receptor was moderate. Unlike 5-HT and NMDA, glycine is an inhibitory neurotransmitter localized in the spinal cord. Blocking the action of glycine causes excitatory event which is not a clinical benefit[18]. While it has been proved through scientific research that mangosteen has various pharmacological activities, its adverse effects on the nervous system have not been reported[19]. Therefore, the excitatory effect of MGTs on glycine receptor might be neutralized by the inhibitory effect of MGTS on 5-HT and NMDA receptors.

The inhibitory activity of pure xanthone (MGT-1 to MGT-7, Fig. 3) on NMDA and glycine receptor was in agreement with that of crude extract MGT-8 suggesting potentiation interaction of each pure xanthone. In contrast, such interaction was not observed in the model of 5-HT receptor.

Considering the difference in the chemical structures, the presence of a 1, 3, 6, 7-tetraoxygenated function comprising a prenyl group at C-8 in the xanthone nucleus might play crucial roles in high-to-moderate potency of MGT-1 and -6 and of MGT-2 and -5 on 5-HT receptor, when compared with no inhibitory activity was observed in MGT-7 which was the 1, 3, 5, 8-oxygenated xanthone with a prenyl unit substitution at C-4. Comparison between the more potency of MGT-1 and -4 with their respective lower potent xanthone analogs MGT-2 and -3 revealed the essence of the methyl ether group at C-7. In addition, the xanthone scaffold bearing a 3-hydroxy-3-methylbutyl moiety at C-8 of MGT-3 and -4 seemed important for such high potency on the NMDA receptor.

In summary, the results revealed that the inhibition of the xanthones was rather selective to different receptors. It is interest to further examine structure-activity relationship to find out the most selective with highest potent xanthone.

What is already known on this topic ?

Xanthones have sedative effects in animals by action on the central nervous system characterised by ptosis, sedation, decreased spontaneous motor activity, loss of muscle tone and potentiation of pentobarbitone sleeping time. However, the mechanism of action underlying these activities has not studied.

What this study adds ?

The sedative effects of xanthones might involve inhibition at one or more stimulatory neurotransmitter receptor (s).

Acknowledgement

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Potential conflicts of interest

None.

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สารแซนโทนจากเปลือกมังคุดยับยั้งตัวรับสารสื่อประสาทที่แสดงออกในไข่กบซีโนปุส

ปัทมา ลิ้วนิช, สุนิตย์ สุขสำราญ

วัตถุประสงค์: เพื่อศึกษาฤทธิ์ยับยั้งของสารแซนโทน 7 ชนิดและสารสกัด 3 ชนิดที่ได้จากเปลือกมังคุดต่อตัวรับซีโรโทนิน เอ็นเอ็มดีเอและไกลซีนที่แสดงออกในไขกบซีโนปุส

วัสดุและวิธีการ: ฉีดอาร์เอ็นเอของตัวรับซีโรโทนิน เอ็นเอ็มดีเอหรือไกลซีนเข้าไปในไขกบซีโนปุส แล้วทดสอบฤทธิ์ยับยั้งของสารแซนโทนและสารสกัดโดยใช้วิธี two-electrode voltage clamp

ผลการศึกษา: สารแซนโทนหลายชนิดสามารถยับยั้งการทำงานของตัวรับซีโรโทนิน เอ็นเอ็มดีเอและไกลซีนได้ด้วยขนาดต่างๆ กัน alpha-mangostin, garcinone-D และ 9-hydroxycalaba xanthone ยับยั้งกระแสไฟฟ้าที่เกิดจากการกระตุ้นด้วยซีโรโทนินมากกว่าร้อยละ 80 gamma-mangostin และ garcinone-E ยับยั้งมากกว่าร้อยละ 50 แต่ไม่เกิน 80 garcinone-C และ garcinone-D ยับยั้งกระแสไฟฟ้าที่เกิดจากการกระตุ้นด้วยกลูตาเมตมากกว่าร้อยละ 80 และ alcohol extract ยับยั้งมากกว่าร้อยละ 50 แต่ไม่เกิน 80 alpha-mangostin, garcinone-C, Garcinone-D, non-tannin extract และ alcohol extract ยับยั้งกระแสไฟฟ้าที่เกิดจากการกระตุ้นด้วยไกลซีนร้อยละ 50-70 ไม่มีสารใดยับยั้งได้ถึงร้อยละ 80

สรุป: ข้อมูลบ่งชี้ว่าอนุพันธ์ของสารแซนโทนมีความสามารถในการเลือกออกฤทธิ์ต่อตัวรับแต่ละชนิดได้แตกต่างกัน