

ANTI-HERPES SIMPLEX VIRUS ACTIVITY OF *SYZYGium JAMBOS*

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ABSTRACT: The hexane, dichloromethane and methanol were extracted from the leaves of *Syzygium jambos* (L.) Alston (Myrtaceae) and investigated for anti-herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2) using plaque reduction assay. Hexane and dichloromethane extracts presented the anti- HSV-1 and HSV-2 activities with more than 50% inhibition of plaque formation at the concentration of 100 µg/ml. The IC₅₀ values of each extract and acyclovir were determined. The cytotoxicity of the extracts to Vero cells was also evaluated. Among all extracts, the dichloromethane extract exhibited the highest activity against HSV-1 and HSV-2 with the IC₅₀ value of 75 µg/ml while it showed the cytotoxicity in Vero cells at the CC₅₀ value of 150 µg/ml. The hexane and methanol also showed the activities against HSV-1 and HSV-2 with the IC₅₀ values of 100 and 300 µg/ml, respectively. The cytotoxicity in Vero cells of hexane and methanol was observed with the CC₅₀ values of 150 and 600 µg/ml, respectively.

Keywords: *Syzygium jambos*, Myrtaceae, Herpes simplex virus, Antiviral agent, Thai medicinal plant

INTRODUCTION: Herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2) are two strains of the herpes virus family Herpesviridae, which causes extremely painful infections in humans¹⁾. HSV-1 is usually associated with infection of the lip, mouth, and face. It is the most common type of herpes simplex virus in which most people develop during childhood. HSV-1 often causes lesions inside the mouth, such as cold sores. It is transmitted by contact with infected saliva. HSV-2 is sexually transmitted. Symptoms include genital ulcers or sores. The virus can also lead to complications such as infection of the lining of the brain and the brain itself (meningoencephalitis) or infection of the eye (especially the conjunctiva and cornea). However, some HSV-2 infected people may not show symptoms. Cross-infection of type 1 and 2 viruses may occur from oral-genital contact²⁾. Nucleoside analogues, one of antiviral products, have been approved for treatment of HSV-1 and HSV-2. The most commonly used is Acyclovir^{3,4)}. Although the effective antiviral agents are available, they are

expensive. Thus, many researchers have sought for the effective and inexpensive anti-HSV agents.

Clinacanthus nutans (Burm.f.) Lindau (Acanthaceae) has been one of the Thai herbs for primary health care, used for the topical treatment of HSV, VZV (varicella-zoster virus) infections and anti-insect bite⁵⁾. Not only the *in vitro* of *C. nutans* extracts for anti-HSV-1, HSV-2 and VZV activities but also the clinical trials were reported for *C. nutans* preparations⁶⁻⁸⁾.

In our continuous work, we examined the HSV-1 and HSV-2 inhibitory activities of Thai medicinal plants. *Syzygium jambos* (L.) Alston (Myrtaceae) is a medicinal plant used in Thailand for fever, diabetic and diarrhea.

S. jambos (syn. *Eugenia jambos*) is a tree with glossy green leaves and white flowers. The edible fruit is shaped like a small pear. There are several varieties, including the one most common in Thailand bearing a pale green fruit. The Thai name is Chom-puu-num-dok-mai⁹⁾.

The present investigation deals with the extraction of the *S. jambos* leaves and examination of the anti-

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HSV-1 and HSV-2 activities of the extracts by using plaque reduction assay. The cytotoxicity of the extracts to Vero cells was also investigated.

MATERIALS AND METHODS:

Plant materials

The leaves of *S. jambos* were collected from Phuket Province, Thailand during March to April, 2006. The plant was identified by one of us (N.R.). The voucher specimen (SWU 131910) was deposited in the herbarium at the Faculty of Pharmacy, Srinakharinwirot University, Nakhon-nayok Province, Thailand.

Plant Extracts

The dried leaves of *S. jambos* (270 g) were successively extracted with hexane (3×3 L), dichloromethane (3×3 L), and methanol (3×3 L). The filtrates were pooled and evaporated under reduced pressure at the temperature not exceeding 40 °C to give the corresponding hexane (5.4 g, 2.0% w/w dry weight of plant material), dichloromethane (4.2 g, 1.6% w/w dry weight of plant material) and methanol extracts (12.5 g, 4.6% w/w dry weight of plant material).

Acyclovir

Acyclovir was purchased from Sigma.

Viruses and cells

Herpes simplex virus type 1 (KOS) and type 2 (186) were used (Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University). Vero cells were cultured in minimum essential medium supplemented with 10% FBS and antibiotic.

Plaque Reduction Assay

Plaque reduction assay was performed by the modified method^{10,11}. Briefly, Vero monolayer cells (6×10^5 cells / ml) in 96-well microtiter plates were infected with HSV-1 (KOS) or HSV-2 (186) (30 plaque forming unit (PFU) / 25 µl). After 1 h of viral adsorption, the overlay medium containing various concentrations of the crude extract was added to the Vero cells and incubated at 37 °C in humidified CO₂ incubator for 2 days. The cells were fixed and stained with 1%

crystal violet in 10% formalin. The plaques were counted and the percent plaque inhibition was determined. The anti-HSV-1 and 2 activities of crude extracts and acyclovir were investigated in terms of 50% inhibitory concentration (IC₅₀). The cytotoxicity was determined by trypan blue exclusion method.

RESULTS AND DISCUSSION:

Three extracts (hexane, dichloromethane and methanol) were isolated from the leaves of *S. jambos* ranging from the least polar (hexane) to the most polar (methanol). In this study we examined the anti-HSV-1 and HSV-2 activities of the extracts by using plaque reduction assay. To determine the anti-HSV-1 and HSV-2 activities, percent inhibition of plaque formation were determined (Table 1). Hexane and dichloromethane extracts at the concentration 100 µg/ml exhibited the anti-HSV-1 and HSV-2 activities with more than 50% inhibition of plaque formation. The IC₅₀ values of each extract and acyclovir were determined. The cytotoxicity of the extracts to Vero cells was also investigated (Table 2). Among all extracts, the dichloro-methane exhibited the highest activities against HSV-1 and HSV-2 with the IC₅₀ value of 75 µg/ml but it possessed the cytotoxicity in Vero cells at the CC₅₀ value of 150 µg/ml.

In 1997, Abad MJ. and co-workers reported that the ethanol extracted from *Eugenia jambos*, Guatemala exhibited the antiviral effect against HSV-1 (at lower concentrations, ranging from 5 to 25 µg/ml)¹² but there was no efficacy against HSV-2.

The results indicate that *S. jambos* is one of the interesting plants to develop for anti-HSV agents.

Table 1 Inhibition (%) of extracts of *S. jambos* against HSV-1 and HSV-2

Extracts*	HSV-1	HSV-2
Hexane	51.3 ±4.8	51.1 ±1.1
Dichloromethane	96.1 ±2.6	94.2 ±0.2
Methanol	11.2 ±3.1	30.6 ±1.4

*Concentration of crude extracts: 100 µg/ml

Table 2 Inhibitory effect of extracts of *S. jambos* against HSV-1 and HSV-2

Extracts	IC ₅₀ (µg/ml)		CC ₅₀ * (µg/ml)
	HSV-1	HSV-2	
Hexane	100	100	150
Dichloromethane	75	75	150
Methanol	300	300	600
Acyclovir	0.34	0.60	-

*50% cytotoxic concentration to Vero cell

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ฤทธิ์ต้านไวรัสเฮอร์ปีซิมเพล็กซ์ของชมพู่น้ำดอกไม้

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บทคัดย่อ: ชมพู่น้ำดอกไม้เป็นพืชในวงศ์ Myrtaceae ในประเทศไทยมีการนำสมุนไพรดังกล่าว มาใช้ลดไข้ รักษาโรคเบาหวาน และแก้ท้องเสีย ในการศึกษาครั้งนี้ได้นำส่วนใบของ ชมพู่น้ำดอกไม้ มาสกัดด้วยตัวทำละลายได้แก่ เฮกเซน ไดคลอโรมีเทน และเมทานอล เพื่อให้ได้สารสกัดจากตัวทำละลายดังกล่าว จากนั้นนำสารสกัดทั้ง 3 ชนิดมาหาฤทธิ์ต้านไวรัสเฮอร์ปีซิมเพล็กซ์ทัยปี 1 และ 2 โดยใช้วิธี plaque reduction assay พบว่าสารสกัดไดคลอโรมีเทนออกฤทธิ์ได้ดีที่สุดในการยับยั้งเชื้อไวรัสเฮอร์ปีซิมเพล็กซ์ทัยปี 1 และ 2 พบว่าสารสกัดเฮกเซน และไดคลอโรมีเทนมีฤทธิ์ยับยั้งไวรัสเฮอร์ปีซิมเพล็กซ์ทัยปี 1 และ 2 โดยยับยั้งได้มากกว่าร้อยละ 50 ที่ความเข้มข้นของสารสกัด 100 µg/ml สารสกัดไดคลอโรมีเทนสามารถยับยั้งไวรัสดังกล่าวได้มากที่สุดโดยความเข้มข้นของสารสกัดที่สามารถยับยั้งเชื้อไวรัสเฮอร์ปีซิมเพล็กซ์ทัยปี 1 และ 2 ได้ร้อยละ 50 (IC₅₀) คือ 75 ไมโครกรัมต่อมิลลิกรัม ซึ่งทำการทดลองเทียบกับยา acyclovir นอกจากนี้ยังพบว่าสารสกัดดังกล่าวมีความเข้มข้นที่เป็นพิษต่อเซลล์ Vero เท่ากับร้อยละ 50 (CC₅₀) มีค่าเท่ากับ 150 ไมโครกรัมต่อมิลลิกรัม นอกจากนี้พบว่าความเข้มข้นของสารสกัดเฮกเซน และเมทานอลที่สามารถยับยั้งเชื้อไวรัสเฮอร์ปีซิมเพล็กซ์ทัยปี 1 และ 2 ได้ร้อยละ 50 เท่ากับ 100 และ 300 ไมโครกรัมต่อมิลลิกรัมตามลำดับ โดยที่ความเข้มข้นที่เป็นพิษต่อเซลล์ Vero เท่ากับร้อยละ 50 มีค่าเท่ากับ 150 และ 600 ไมโครกรัมต่อมิลลิกรัมตามลำดับ

คำสำคัญ: *Syzygium jambos*, Myrtaceae, ไวรัสเฮอร์ปีซิมเพล็กซ์, ฤทธิ์ต้านไวรัส, สมุนไพรรักษา

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