Anti-herpes simplex virus type 2 activity from *Rhinacanthus nasutus* (Linn.) Kurz. extracts as affected by different extraction solvents

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https://doi.org/10.12982/CMUJNS.2019.0002

Received: *April 1, 2018*
Revised: *June 21, 2018*
Accepted: *July 13, 2018*

ABSTRACT

Rhinacanthus nasutus (Linn.) Kurz. is a medicinal plant traditionally used in northern Thailand. It has anti-bacterial, anti-inflammatory, anti-cancer, and anti-viral properties. This study investigated the anti-herpes simplex virus type 2 (anti-HSV2) activity of stem extracts of R. nasutus (Linn.) Kurz. on American green monkey kidney (or Vero) cells. The plant extracts were prepared with six different solvents: ethanol, ethylacetate, methanol, dichloromethane, acetone and hexane. The cytotoxicities of the stem extracts were determined using MTT assay with various concentrations of the stem extracts (2.44 to 156 µg/mL) and the 50% cytotoxicity dose (CD_{50}) was calculated after the Vero cells were treated with the stems extracts for 24 h. The methanolic extract showed a low cytotoxicity on the Vero cells, with a CD_{50} value of 78.22 ± 23.54 µg/mL; in contrast, the ethylacetate, dichloromethane, acetone and hexane extracts were highly toxic to the Vero cells. The anti-HSV2 activities of the plant extracts were investigated by plaque reduction assay after treating the infected Vero cells for 24 h with the stem extracts at concentrations between 2.44 and 19.50 µg/mL. The inhibitory effect values of the 50% effective dose (ED_{50}) were determined. The methanolic extract had the highest inhibitory effect (ED_{50} of 16.16 + 10.83 µg/mL) against HSV-2 infections and inhibited HSV-2 particles by 70.5% after 24 h of

treatment. Our results showed the potential for using methanolic extracts from the stem of R. nasutus (Linn.) Kurz. to treat HSV2 infections during the late stage viral life cycle 24 h after infection.

Keywords: Anti-herpes simplex virus type 2 activity, Cytotoxicity, *Rhinacanthus nasutus* (Linn.) Kurz.

INTRODUCTION

Herpes simplex virus (HSV) is a single, large, double-stranded DNA virus that infects humans; it is widespread among the world's population, including in Thailand. Both herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are members of the family *Herpesviridae*. HSV infects and replicates in human cells at the site of entry, especially mucocutaneous surfaces. The virus infects into the cell bodies of neurons in the sensory ganglion, damaging the infected sensory neurons. HSV-1 infection is a common cause of infections of the oral facial mucosal surfaces. HSV-2, or genital herpes, is the most common cause of genital ulcers (Yoosook et al., 1999; Yang et al., 2005; Koch et al., 2008). HSV is also an important cause of neonatal infections (Schomogyi et al., 1998; Reynolds et al., 2003; Straface et al., 2012). The HSV-2 genome is separated into long and short regions of unique sequences, termed U_L and U_S (Dolan et al., 1998; Taylor et al., 2002; Antrobus et al., 2009). The 155 kb genome of HSV-2 consists of two regions of unique sequences with a G+C content of 70.4%. The viral DNA encodes 74 gene products (Dolan et al., 1998).

Acyclovir (ACV) and other nucleoside derivatives, such as penciclovir, famciclovir, valaciclovir and ganciclovir, have been approved for HSV-1 and HSV-2 treatment. However, resistance to acyclovir and its analogs can occur following mutation in either HSV thymidine kinase or DNA polymerase, although the emergence of acyclovir-resistant strains of HSV appears to be infrequent in immunologically normal individuals (Whiteley et al., 1998; Stanberry et al., 1999). The potential for resistant strains and the high cost of treating HSV have led to seeking alternative treatments using medicinal plants. Many medicinal plants that have been shown to contain anti-herpes simplex viral activities, and used in traditional herpes treatments, are indigenous to Southeast Asia, including Thailand; these include *Punica granatum, Ocimum sanctum, Azadirachta indica, Nyctanthes arbortristis, and Rhinacanthus nasutus* (Linn.) Kurz. (Bourne et al., 1999; Yoosook et al., 1999; Yoosook et al., 2000; Yang et al., 2005; Xiong et al., 2011; Nakama et al., 2012). We focused on *Rhinacanthus nasutus* (Linn.) Kurz. due to its therapeutic effects, including anti-fungal, anti-bacterial, anti-tumor, hepatoprotective and anti-inflammatory activities (Kumar et al., 2012). *R. nasutus* (Linn.) Kurz is known as *Thong Pan Chung* in Thai. It is a small slender shrub, erect, branched and 1-2 m in height. *R. nasutus* (Linn.) Kurz. is widely spread across sub-continental Asia, including parts of India, China and Southeast Asia. The phytochemistry and biology of *R. nasutus* (Linn.) Kurz. have been extensively investigated. Major constituents include flavonoids, steroids, terpenoids, anthraquinones, lignin groups and, especially, napthoquinones, such as rhinacanthins (A-D, G-Q) (Suman et al., 2011). The objective of the current study was to evaluate the cytotoxicity and anti-HSV-2 activity of stem extracts from *R. nasutus* (Linn.) Kurz. using ethanol, methanol, ethylacetate, dichloromethane, acetone and hexane as extraction solvents on an American green monkey kidney cell line (Vero cell) model.