CHAPTER I
INTRODUCTION

1. Rationale and background

Dengue virus (DV) infection is an important public health problem because it is a serious cause of morbidity and mortality in human. It is usually characterized by acute high grade fever but more serious syndrome such as dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS) sometimes occurr following a dengue infection which is usually the result of sequential infection with multiple serotypes [1]. Most DV infections are asymptomatic or it can cause a nonspecific viral syndrome. Few of them suffer dengue fever (DF) that is almost always a self-limited. The WHO estimates that 50–100 million individuals contract DF each year; of these, 500,000 people suffer from DHF and DSS with approximately 20,000 deaths [2]. Presently, all four serotypes of DV co-circulate most in tropical and subtropical areas of the world in over 100 countries. The mechanisms involved in the disease pathogenesis are not well understood and are still a challenge. The severity of clinical presentation seems to result from both viral factors and host immune response [3]. Infection with one dengue serotype may provide lifelong immunity to that serotype, but there is no cross-protective immunity to other serotypes [4].

Secondary infections by a different serotype are much more likely to lead to DHF and DSS than primary infections and the possibility to immunize against one serotype might put a person at risk for a more serious illness. Therefore, the development of vaccine against DV has progressed slowly. Currently, studies of molecular level of viral genome structure and replication mechanism make the scientists to understand the mechanism of DV replication cycle in host cells and are useful for development of drug and vaccine.

At present, there is no effective therapeutic treatment for DV infection. DV vaccine has also been challenging to develop for vaccine that can simultaneously immunize and induce a long-lasting protection against all four serotype of DV. An incompletely immunized individual may be sensitized to life-threatening DHF or

DSS. Hospital treatment in general is given as supportive care which includes bed rest, antipyretics, and analgesics. Therefore, there is a requirement for effective anti-viral agents and therapeutic concepts for DV infection [5]. Several types of anti-viral agent have been sought intensively, including inhibitors against viral replication, posttranslational processing of viral proteins and E protein functions such as membrane fusion and virus attachment. Blocking of virus attachment or entry into host cells is an effective strategy to control virus infection. This type of inhibitor, termed an entry inhibitor, blocks structural rearrangements of the viral E that are essential for viral infection [5, 6].

The developments of drug from medicinal plants have been widely used to treat a variety of infectiou illnesses. Several of these may have been used to treat viral infections. The molecular mechanism associated with the anti-viral effects of plant extract may vary among different virus. Additionally, the potentials of plant extract to boot inherent anti- viral defense of human body which involves an intricate immune system might utilize common pathways. Recently, a number of studies have explored immunostimulatory properties of plant extracts that have anti-viral properties.

Dengue pathogenesis is not completely understood, and the main determinants of the developments of severe forms are not yet well established. Most DHF and DSS occur following a secondary infection or an infection with a more virulent virus. Two principal hypotheses explain this epidemiological pattern: (1) the immune enhancement theory maintains that hemorrhage occurs in secondary infections when DV-specific antibodies and memory T cells from primary infection with a different serotype enhance the binding of heterologous DV–IgG complexes to Fcg receptors on monocytic cells; (2) the virulence hypothesis suggests that some DV strains are intrinsically more virulent than others, and cause higher viremia leading to more severe disease [7]. Increase in capillary permeability associated with endothelial activation and haemorrhagic phenomena are landmarks of severe clinical manifestations, strongly suggesting an alteration in immune-regulation.

Cytokines are proteins secreted during innate and adaptive immunological responses, acting as inflammatory mediators or modulatory molecules during several haemorrhagic fevers. Clinical studies support a key role for cytokines and

prostaglandin E2 (PGE_2) in the DHF pathogenesis. During DV infections, cytokines are involved in the disease onset and homeostatic regulation. This activation is more striking in patients with severe clinical manifestations, although it can be found at lower degrees in patients with mild disease.

Most virus infections induce cycloxygenase-2 (COX-2) expression which is regulated by numerous transcription factors, including nuclear factor-kappa B (NF-κB), nuclear factor of activated T cells (NFAT)/activator protein-1 (AP-1). COX-2 is the rate-limiting enzyme for catalysis of arachidonic acid to prostaglandin G2 that further reduced to prostaglandin H2 (PGH_2) and is precursor of various prostanoids, namely prostaglandins (including PGE_2, prostacyclins and thromboxanes. Overexpression of COX-2 leads to increase levels of PGE_2 that is one of the most important pro-inflammatory cytokines and associated with induction of inflammation, leukocyte chemoattraction, pain perception and participates in a wide range of normal physiological processes. PGE_2 regulates viral replication and virulence. However, some reports showed that a level of PGE_2 might enhance viral replication and did not have inhibition effect [8, 9].

Modulation of COX-2/PGE_2 synthesis in stimulated cells by anti-inflammatory molecules could be away to suppress viral replication and spread. Non-steroidal anti-inflammatory drugs, potent nonselective COX inhibitors and pain relievers like aspirin, indomethacin and ibuprofen, all have been shown to exert anti-viral effects or attenuate disease severity during infection by herpes simplex virus (HSV).

Both *Clinacanthus nutans* and *Andrographis paniculata* compounds derived from two commonly used Thai herbs contain properties of anti-viral infection and immunomodulation. Therefore, this study proposed to determine the potential anti-viral effects of compounds from *C. nutans* and *A. paniculata* on DV2 infection and induction of COX-2 expression and PGE_2 production. In addition, the inhibitory effect of PGE_2 on DV replication was also determined.

2. The objectives of thesis

1. To determine effects of compounds from *C. nutans* and *A. paniculata* on DV2 infection in A549 cell line.
2. To determine mechanisms of compounds from *C. nutans* and *A.paniculata* on inhibition step of DV2 infection.
3. To investigate effects of compounds from *C. nutans* and *A. paniculata* on PGE_2 production in DV2 infection.

3. Scope and limitations of research

1. Investigation of the concentration of compounds from *C. nutans* and *A. paniculata* that exhibit non-cytoxicity on cell culture.
2. Screening of anti-DV2 activity of compound from *C. nutans* and *A. paniculata.*
3. Studying of effect of compounds from *C. nutans* and *A. paniculata* on DV2 infection and immunomodulating on COX-2 expression and PGE_2 production.

4. Research site

All processes were performed in microbiology laboratory, Department of Microbiology, Faculty of Medicine, Khon Kaen University.

5. Anticipated outcome

1. Information about the effects of compounds from *C. nutans* and *A. paniculata* on DV2 infection.
2. Information about the mechanisms of compounds from *C. nutans* and *A. paniculata* on inhibition step of DV2 infection.
3. Information about the effects of compounds from *C. nutans* and *A. paniculata* on PGE_2 production in DV2 infection.