CHAPTER 2

LITERATURE REVIEW

1. Viruses

Viruses are small infectious obligate intracellular parasites. Virus genomes compose of DNA or RNA, which are directly synthesis virion components. Some viral species may be surrounded by protective, virus-coded protein envelope (Fleming *et al*., 1997). Viruses have numerous invasion properties. Each strain of virus is enabling to enter into hosts by precisely binding to specific receptor molecules of target cells on surfaces membranes. The virus was de-enveloped before entry into the cytoplasm of host cell and followed by viral replication cycle (Jassim and Naji, 2003).

2. Human herpesviruses

Herpesviruses consist of more than eighty viruses and eight of them are known as human pathogens, which can cause a range of human infectious diseases. Human herpesviruses belong to three groups; alphaherpesvirus, betaherpesvirus and gammaherpesvirus. Alphaherpesvirus are neurotropic and have rapid replication cycle, and broad host range, while betaherpesvirus and gammaherpesvirus show slowly replication and have different in genome size and structure. These different types of human herpesvirus are divided based on genome structure, target cell, pathogenesis, reproduction cycle and site of latent infection (Fatahzadeh and Scwartz, 2007). Human herpesvirus are shown in Table 1.

Table 1 Properties of human herpesviruses (Hunt, 2013; Penkert and Kalejta, 2011)

Subfamily	Virus	Target cell	Site of latency	Transmission
Alphaherpesvirinae				
Human herpesvirus 1	Herpes simplex type 1 (HSV-1)	Mucoepithelial cells	Neuron	Close contact
Human herpesvirus 2	Herpes simplex type 2 (HSV-2)	Mucoepithelial cells	Neuron	Close contact by sexual activity
Human herpesvirus 3	Varicella-zoster virus (VZV)	Mucoepithelial cells	Neuron	Respiratory route or physical contact
Gammaherpesvirinae				
Human herpesvirus 4	Epstein-Barr virus (EBV)	B lymphocyte and epithelial cells	B lymphocytes	Saliva via kissing
Human herpesvirus 8	Kaposi's sarcoma-related virus (KSHV)	Endothelial cells	Unknown	Saliva and body fluids

Table 1 (continued)

Subfamily	Virus	Target cell	Site of latency	Transmission
Betaherpesvirinae				
Human herpesvirus 5	Cytomegalovirus (CMV)	Monocyte, epithelial, and cells lymphocyte	Monocyte, lymphocyte and possible others	Close contact, congenital blood transfusion and tissue transplantation
Human herpesvirus 6	Herpes lymphotropic virus	T lymphocyte and others	T lymphocytes and others	Respiratory route and close contact
Human herpesvirus 7	Human herpesvirus 7 (HHV-7)	T lymphocyte and others	T lymphocytes and others	Unknown

3. Herpes simplex virus

3.1 Background and biological properties

Herpes simplex virus (HSV) infectious diseases occur worldwide and produce serious illnesses. The virus is responsible for a wide variety of common human infection, which is easily transmitted in both developed and developing countries. The first recognized of herpes occur in ancient Greek. The word herpes itself derived from "*herpein*" that means to creep or crawl and describes spreading and characteristic nature pattern of the skin lesion caused by herpes simplex virus. HSVs are classified to genus *Simplexvirus*, a member of subfamily *Alphaherpesvirinae*, family *Herpesviridae*. This classification is based on their biological properties, common structure and location in latent state (Albà *et al.*, 2001; Gupta *et al*., 2007; Pereira *et al*., 2012).

Herpesviridae is human pathogenic viruses and causes major illness of morbidity and mortality. HSVs use the host cell machinery and directly transcript viral gene. HSV infection has a short replication cycle and the virus has ability to destroy infected cells with a variety of diseases (Armaka *et al*., 1999; Taylor *et al*., 2002). Additionally, HSV infection produces clinical symptoms ranging from mild to serves disease. Primary infection causes vesicular lesions in the mucosal epithelia cells. Viruses are able to spread and establish latent infection in host cells within sensory neurons. Recurrent disease is usually present at or near the site of primary infection (Mandal *et al*., 2008; Tolo *et al*., 2006).

Herpesviruses compose of two serotypes, which are herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). HSV-1 and HSV-2 are similar in tissue tropism and DNA sequence. Polymerase chain reaction (PCR) technique shows the sequence

homology of both HSV-1 and HSV-2 approximately 85% similarity (Arama *et al*., 2010; Riley, 1998; Whitley and Roizman, 2001). Therefore, different remarkable between two viral subtypes are also classified by their location in the latent state, epidemiology, DNA homology, antigenic determinants, and symptom of disease (Brugha *et al*., 1997; Lucotte *et al*., 1995; Taylor *et al*., 2002). Therefore, HSV-1 and HSV-2 are closely related in clinically indistinguishable and share many common epitope and characteristics, which are resulting in cross-reactive responses in serological assays (Whitley *et al*., 1998).

Both types of HSV infections cause chronic disease and affect populations worldwide in both rural and urban. It is life-long infections with intermittent clinical and subclinical viral reactivation and shedding from mucosal surfaces, which is depended upon the type involved (Lückemeyer *et al*., 2012; Madhavan *et al*., 1999; Sierra *et al*., 2011).

3.2 Structure properties of herpes simplex virus

HSV is a diverse group of a large double-stranded linear DNA molecule and the diameter of virion particle is approximately 150-200 nm in size. HSV particle consists of nucleotide with 32-75% G+C, depending on the virus species (Spear and Roizman, 1972). DNA core is packed in an icosahedral capsid that coated with a layer of proteins called tegument and enclosed with a glycoprotein-containing envelope. The envelope protects viral genome from various conditions in extracellular environment. Moreover, several glycoproteins in envelope are essential for viral particle fusion and entry into infected cells (Spear, 2004).

Four structural features of HSV particle are as follows.

(1) Viral genome

Structure of viral particle consists of a central opaque dense core containing viral genome in form of torus structure encoding about 100-200 genes that is packaged tightly in an icosahedral capsid. Inner core is surrounded by an envelope, which is derived from cellular membranes (Figure 2) (Furlong *et al.*, 1972; Karasneh and Shukla, 2011; Spear, 2004).

The HSV genome is a single large double-stranded linear DNA molecule. It consists of approximately 150,000 base pairs and encodes at least approximately 80 proteins. However, only half of these proteins are necessary for directly control of viral replication and facilitate interaction between virus and different host cells while other proteins have function on interaction with host cell or immune response (Taylor *et al.*, 2002).

Viral genome and their protein products are generally composed of two covalently linked segments, called long (L) or short (S) regions, which are based upon their relative length. Each segment contains unique sequence; U_L (unique long) and U_S (unique short) region that are flanked by large inverted repeats (Figure 1) (Taylor *et al.*, 2002).

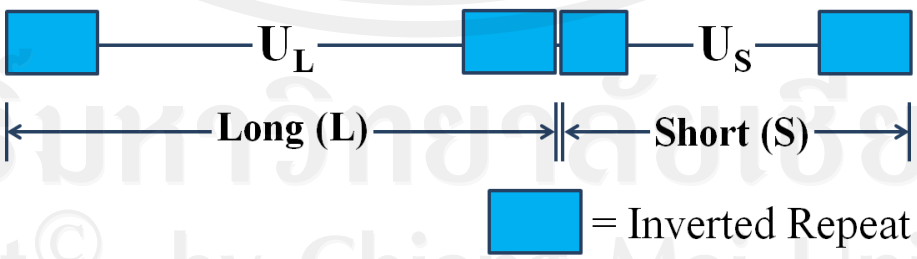

Figure 1 HSV genome organizations (modified from Taylor *et al.*, 2002)

Viral genome is released into the nucleus for viral gene expression. HSV genome encodes several numbers of essential enzymes, which are DNA-dependent DNA polymerase and other enzyme such as thymidine kinase, ribonucleotide reductase, serine-protease and protease. Thus, these enzymes and DNA polymerase are suitable targets for antiviral treatment (Figure 2) (Simonato *et al*., 2000; Taylor *et al*., 2002).

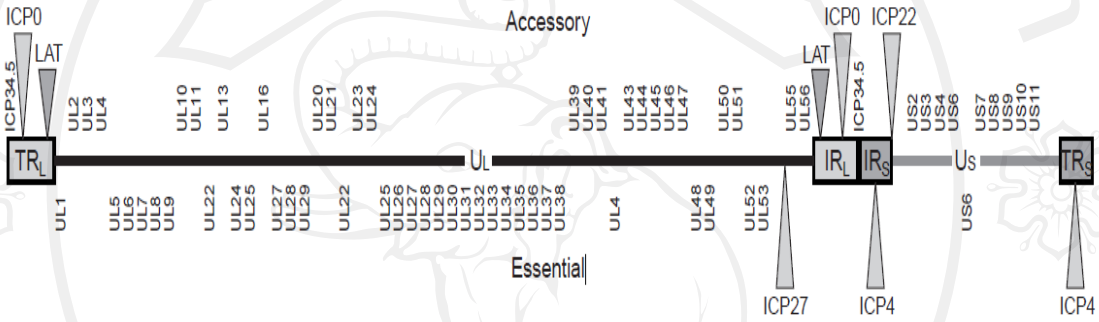

Figure 2 HSV genome (Simonato *et al*., 2000)

(2) An icosahedral capsid

HSV genome is packed within an icosahedral capsid and surrounded by a membrane that is derived from host nuclear membrane. Major capsid shells are 125 nm in diameter thick and icosahedral structure. Capsid protein displays 162 capsomers, which composed of 12 pentons and 150 hexons (Davison, 2010; Spear, 2004). The pentons and hexons are connected together by 320 triplexes and triplex lies at local three-fold position created by a group of three capsomers (Baines, 2011). In addition, HSV composes of 3 capsid types, which are A, B and C-capsids. All of them have same structure but only C-capsid contains DNA and it is able to mature into infectious virus (Figure 3) (Brown and Newcomb, 2011; Spencer *et al*., 1997).

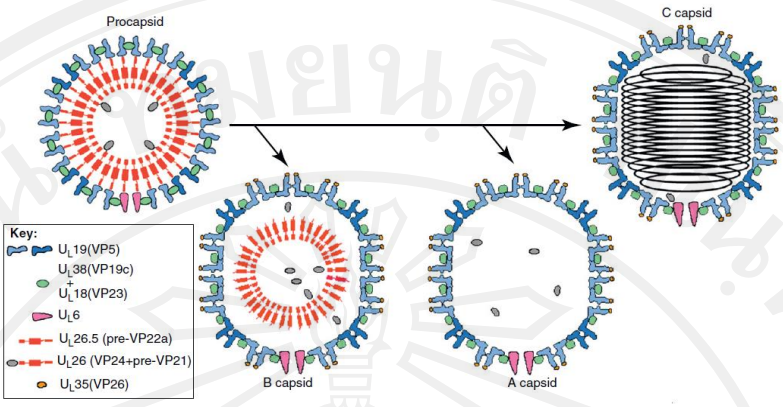

Figure 3 Structure of HSV-1 maturation (Baines, 2011)

(3) A tegument

Tegument is an amorphous layer of proteins surrounded the capsid. The space of tegument is between envelope and capsid, which contains viral proteins and enzymes that are essential for replication. Therefore, this component contains various viral proteins (Figure 4) (Foster *et al*., 1998; Kelly *et al*., 2012).

(4) Envelope

Envelope or outer membrane of mature HSV is derived from portions of inner lamella nuclear membrane and cisternae of endoplasmic reticulum of infected cell. Viral particles move from nucleus into cytoplasm. Virus exits cytoplasmic membranes of the cell by budding into vesicles derived from Golgi complex and contains viral glycoprotein spikes. Therefore, virus that has fragile membrane and damage envelope is not infectious. The infectious virus can be transmitted by direct contact with infected mucosal membrane or infections secretions from infected person (Spear and Roizman, 1972).

Herpesviruses have several viral-encoded glycoproteins embedded on their surface envelope. Major function of HSV envelope and envelope glycoproteins are

important for interactions with host cell receptors to promote entry and fusion to the cells, and escape viral immune mechanisms by binding complement or a portion of the antibody (Riley, 1998; Sarmiento and Spear, 1979). Normally, enveloped viruses are sensitive to acid, detergent, dry, organic solvent and also potential target for antiviral agents (Figure 4).

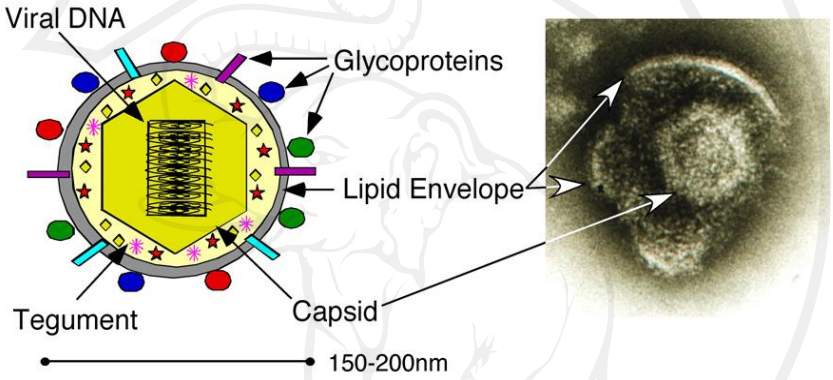

Figure 4 HSV structure composes of viral DNA, icosahedral capsid, tegument and envelope (Taylor *et al*., 2002)

4. Herpes simplex virus infection

Initial of infection

Primary infection occurs when individual is infected with either HSV-1 or HSV-2. This initial infection begins with HSV entry to the body through epithelial or mucus membrane by attachment with specific cell-surface receptor of host cell. Then, the virus fuses its envelope with cellular membrane leading to viral invasion of epithelial cells. After that, virus transports capsid through nuclear pore and follows by releasing viral DNA into nucleus to begin intracellular cytolytic replication (Whitley and Roizman, 2001). These infection leads to damage the infected cells

because HSV induces epithelial and dermal cells detachment, cell fusion and intranuclear inclusions. The lesions of fluid-filled blisters containing cellular debris, inflammatory cells, and progeny virions are found (Gupta *et al*., 2007).

Therefore, primary symptoms of HSV infections cause "flu-like" syndrome, which the patients are present fever, headache, malaise, diffuse myalgia follow by local symptoms consisting of itching, tenderness, dysuria lesions, painful and ulceration. Viral shedding occurs during primary infection or during subsequent recurrences leading possible transmission. Moreover, people with asymptomatic infections also shed the virus (Khan *et al*., 2005).

Latent infection

After primary infection, viral genomes in form of circular extra-chromosomal DNA are transported through periaxonal sheath of sensory nerves to nuclei of neuronal cell bodies of host nervous system that connect the point of entry body. The viral genome is maintained to establish life-long in sensory neurons of the peripheral nervous system in the state of latency. The virus can be reactivated episodically. Major reservoir of latent infections is sensory neurons in ganglion tissue, either trigeminal ganglion for HSV-1 or sacral lumbosacral ganglia for HSV-2 to persist in a dormant state for life. Thus, virus remains hidden in the cell by avoiding immune surveillance (Fatahzadeh *et al*., 2007; Piret and Boivin, 2011; Spear and Roizman, 1972).

The characteristic of this latent infection demonstrates that the viral genome remains in tissue without production of infectious viral particle to damages tissue. The presence of virus can be detected by immunofluorescence microscopy using antibodies against HSV immediately early protein (Figure 5) (Lachmann, 2003;

Steiner and Kennedy, 1995). During latent period, viral DNA and RNA transcripts can be detectable but viral-encoded proteins are not produced. However, RNA transcripts that are produced during latency are important in reactivation of HSV from the latent state (Riley, 1998; Taylor *et al.*, 2002).

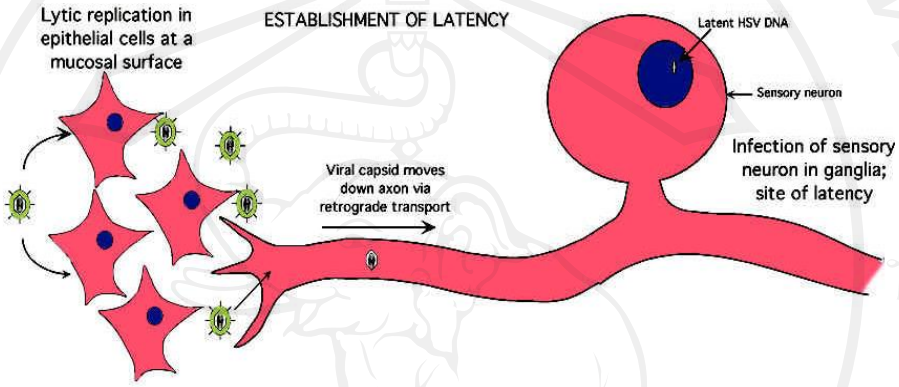

Figure 5 Stages of HSV initial infection (Taylor *et al.*, 2002)

Reactivation and recurrent infection

HSV reactivation from latency is an essential element of herpesvirus life cycle. This process maintains the potential to reactivate episodes of replication and symptoms that occurs at the same site of primary infection by promoting viral replication in the nerve and leads to spread the viral infection to new host. However, reactivation of latent virus depends on an intact anterior nerve route and peripheral nerve pathways. Moreover, subclinical HSV infection depends on site, time and stage of immune system (Figure 6).

The sporadic reactivation of latent viral gene expression from establishment latency is generally triggered after response to variety of internal factors; physical properties and psychological stress such as fatigue, febrile illnesses, tissue damage,

menstruation, sexual intercourse, temperature, ultraviolet irradiation, corticosteroid administration, laser surgery and nerve damage (Piret and Boivin, 2011; Taylor *et al*., 2002; Whitley and Roizman, 2001). Theses stimuli can reactivate the virus from neuron to move along the nerve from sensory ganglia to give renewal of proliferation of activated virus. The recurrent infections lead to clinical disease on the skin that involve mucosal membrane or squamous epithelial areas to cause lesions of vesicles cluster in the vicinity of the initial site of infection (Fatahzadeh and Schwartz, 2007; Itzhaki *et al*., 1997; Lachmann, 2003).

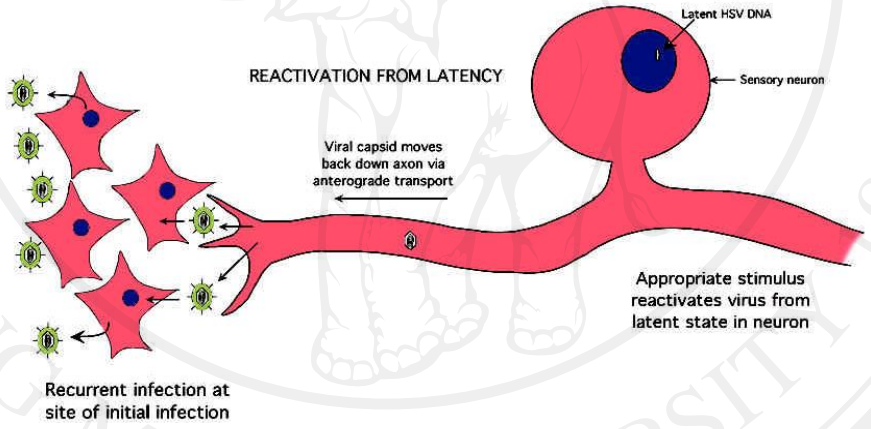

Figure 6 Stages of HSV reactivation (Taylor *et al*., 2002)

Viral reactivation can produce recurrent symptomatic and asymptomatic infection. HSV can be reactivated causing frequent recurrent infections in some patients while most people experience few recurrences (Khan *et al*., 2005; Spear, 2004).

Therefore, the diseases of HSV reactivation from trigeminal ganglion generally occur on oral and nasal mucosa, which are the border of the lip to produce infectious vesicles while lesion around genital area is caused by reactivation of HSV

from sacral nerve ganglia. Moreover, clinical presentation of recurrent infection is generally less stringent, more localized, and shorter time of symptom than primary infection because of the immune responses is expressed (Figure 7) (Doherty *et al.*, 2010; Spear and Roizman, 1972).

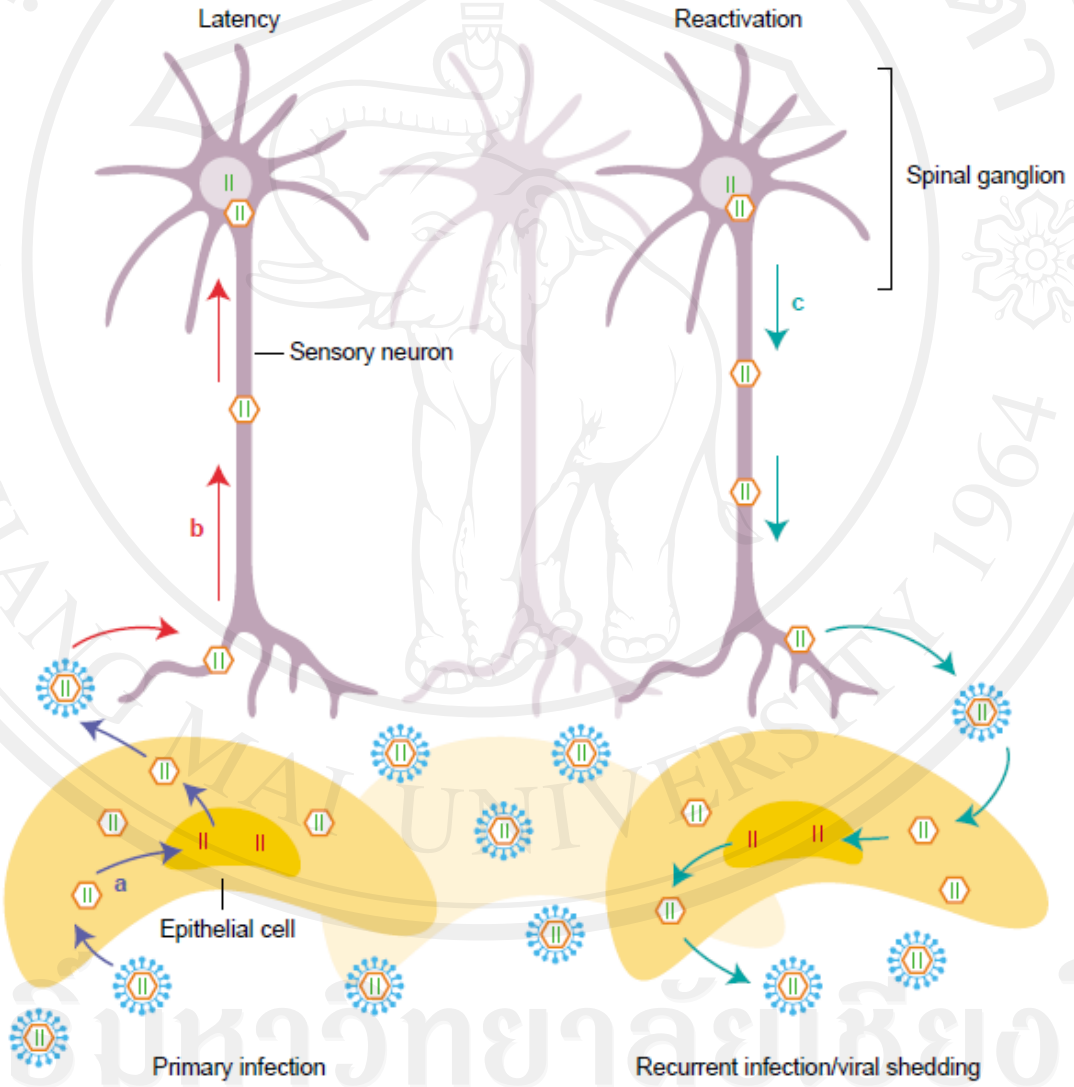

Figure 7 Primary and recurrent of HSV infection (Lachmann, 2003)

5. Herpes simplex virus attachment and entry

HSV infections are composed of attachment and entry to infected cell. Then, replication and expression of viral DNA are occurred after viral uncoating in nucleus. After that, nucleocapsids are assembled and exited from epithelial cells to cause a primary infection. Besides, some of viruses enter sensory neuron and travel retrograde to the nucleus where the viruses establish latency depending on type of HSV. Moreover, reactivation results from anterograde transport of viral particles by viral shedding from the neuron, and re-infection of epithelial cells at initial site of infection (Lachmann, 2003).

Replication of HSV occurs when viral enter into the susceptible host cell. Herpes simplex virus is able to replicate in many cell types and leads to infected cells changes from necrosis, intranuclear inclusion body, swelling with condense chromatin, nuclear degeneration and formation of multinucleated giant cells together with inflammatory response (Taylor *et al.*, 2002; Whitley and Roizman, 2001).

Entry of HSV into host cells acquires interaction between multiple HSV glycoproteins (g) components on surface of viral envelope with multiple specific cell surface receptor molecules on host cell surface. The important target receptors for HSV infection are epithelial cell, mucosa, neurons and leucocytes.

HSV encodes surface glycoproteins more than 11 known viral glycoproteins (B-M) including gB, gC, gD, gE, gF, gH, gI, gK, gM and gN. gB, gC, gD and gH are required for attachment to cell surface. Virus initially attaches to host cell by interaction process between virion gC and herpanran sulfate (HS) proteoglycan on the host cell surface. HS is a ubiquitous glycosaminoglycan (GAG) found on cell membrane and basement membrane of many cells type (Hung *et al.*, 2002). However,

the viral attachment to only HS does not enable entry to host cell. Then, gD protein interacts with one or more cellular co-receptor molecules on cell surface such as herpes virus entry (Hve) protein and leads to the stability of virion attachment. After that, gD conformation is changed and interaction with gH/gL herterodimer on viral surface triggers the fusion step of viral envelope and plasma membrane, and HSV nucleocapsid penetrates to host cell (Figure 8) (Frampton *et al.*, 2005; Hung *et al.*, 2002; Spear, 2004).

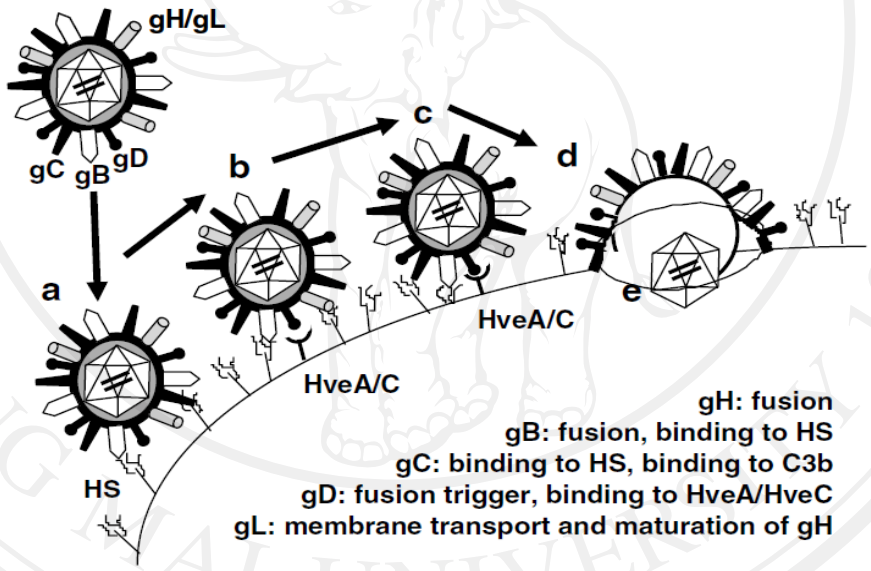

Figure 8 HSV-1 entries into host cell (a) Interaction binding between gC and gB with HS on host cell surface (b) Diffusion of cell surface to HveA/HveC receptor (c) gD binding with HveA/HveC receptor (d) Fusion viral envelope and cell membrane (e) Capsid protein is released into cytoplasm (Frampton *et al.*, 2005)

HSV co-receptors are divided into three structural families. First, herpesvirus entry mediator (HVEM) or HveA is a member of the tumor necrosis factor (TNF) receptor family (Spear and Roizman, 1972). Second, members of the poliovirus receptor related (PRR) immunoglobulin superfamily include HveB, HveC (nectin-1α), and HIgR (nectin-2α). Finally, specific site in heparan sulphate is 3-O-sulfated heparan sulfate (3-O-HS) (Figure 9) (Banfield *et al*., 1995; Mardberg *et al*., 2002; Montgomery *et al*., 1996). Additionally, it also binds to the C3b component of complement system and can block complement-mediated neutralization of virus. gB also interacts with HS or chondroitin sulfate (CS) proteoglycans (Bender *et al*., 2005; Spear, 2004).

Furthermore, gE and gI are structural proteins and immune escape proteins. gE is required for basolateral transmission of virus in polarized cells and efficient expression of late genes (Whitley *et al*., 1998). The other glycoproteins as well as several nonglycosylated membrane associated proteins have function in several important roles such as virus entry via fusion of viral envelope with cellular membrane, intracellular virion morphogenesis, egress and cell-to-cell spread (Melancon *et al*., 2005).

HSV-1 and HSV-2 have the different in some receptors. Both HVEM and nectin-1 are entry receptors for both serotypes while nectin-2 is virtually inactive for HSV-1 entry and weak entry for HSV-2. Moreover, glycoproteins gB, gH and gL are structurally conserved among all herpessviruses and probably have essential roles in viral entry. On the other hand, glycoproteins gC and gD are conserved among most of the neurotropic alphaherpesviruses but do not have recognizable structural

homologues in members of the other two genus of the herpesvirus family (Spear, 2004).

Therefore, HSV can also entry through host cell by multiple steps that results from fusion viral envelope with host cell by pH independent fusion with plasma membrane and further fuses into acidic, or neutral endosomes, or macropinocytosis pathway (Figure 10). In addition, HSV glycoprotein can fuse with host membrane at neutral and low pH. However, specific pathway of HSV entry into host cell differs and depends on upon various cells type-specific (Campadelli-Fiume *et al*., 2012; Karasneh and Shukla, 2011).

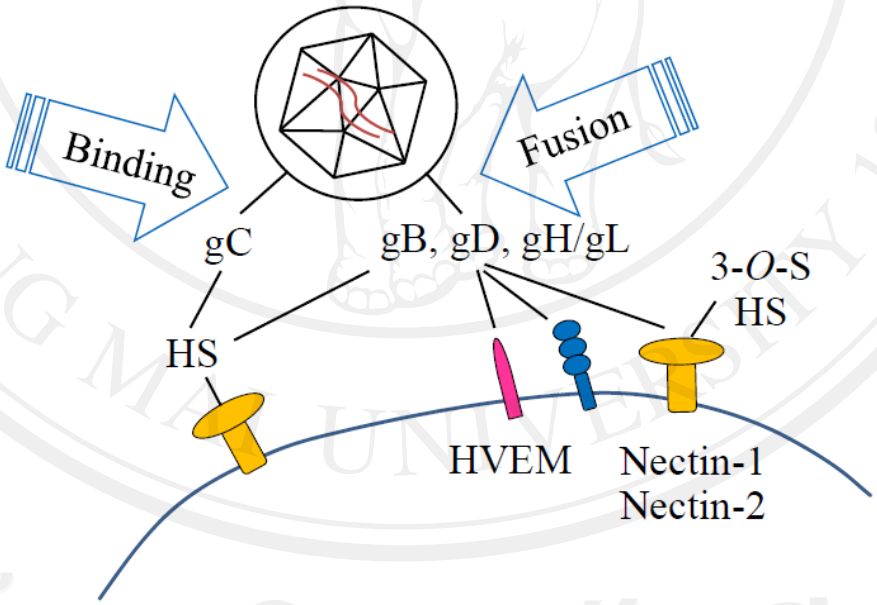

Figure 9 Cell surface receptors and viral glycoprotein requirement for HSV attachment and entry (modified from Spear, 2004)

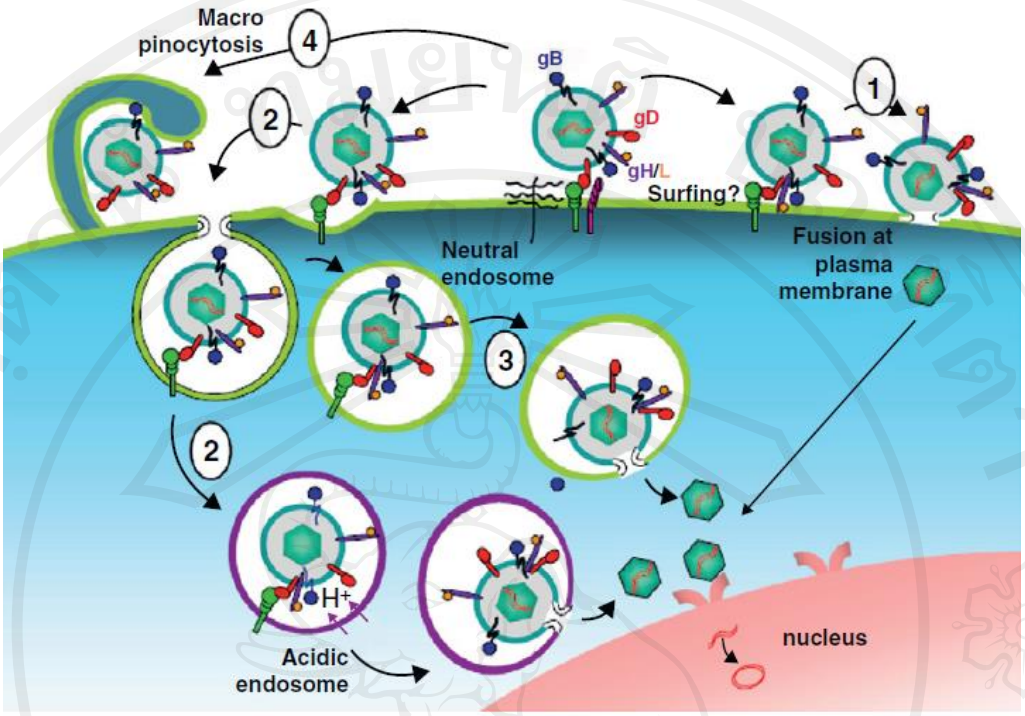

Figure 10 Diversity of herpes simplex virus entry pathway (1) Fusion at cell membrane (2) Endocytosis into acidic or (3) Neutral endosome (4) Macropinocytosis (Campadelli-Fiume *et al*., 2012)

After HSV successful entries and penetrates into host cell by fusion of viral envelope with cellular membrane of host cell. Then, viral nucleocapsid and some tegument proteins (VP16, VP1-2) are transported through cellular microtubules network via nuclear pore into the nucleus following by viral DNA, which is released into nucleus whereas other tegument proteins such as the host shutoff protein (vhs, U_s11) remain in cytoplasm. This process causes disaggregation of polyribosomes and degradation of cellular DNA. Therefore, viral gene expression and replication occur in nucleus and viral assembly occurs in cytoplasm of the host cell (Whitley *et al*., 1998).

6. Replication and expression of herpes simplex viral DNA

Herpesvirus replication is occurred in neuronal nucleus. HSV DNA genome enters nucleus through nuclear pore via microtubules. After DNA replication, circular concatameric forms of HSV genome are made by a rolling circle mechanism.

Transcription of viral genome and viral protein synthesis occur in three sequential phases; immediate early (α), early (β) and late (γ) genes (Figure 11) (Preston *et al*., 1988). VP16 stimulates transcription of immediate early genes (IE) or alpha gene with transcription about 2-4 hours after infection. The alpha proteins consist of DNA-binding proteins, transcription factors and enzymes that are assigned to lytic infection by regulation of early and late gene expression. Moreover, IE gene also stimulates transcription of the early gene (β) synthesis. The early gene (E) or beta gene is expressed at 4-8 hours to 15 hours after infection. The early gene products compose of DNA polymerase for initial replication of viral DNA and enzymes that degrade cellular mRNA and proteins. Moreover, other early gene products are required for DNA replication and act as transcription factors. Circular concatemers are made and then switch to linear chains of individual molecule that are cleaved into monomers by a rolling circle mechanism. Finally, viral DNA replication stimulates the expression of late genes (L) or gamma gene, which encode structural proteins after 12 hour of viral infection. This product is mainly structural and additional proteins of virion. The proteins assemble to form the capsid and tegument, and they are also incorporated into nuclear membranes to form envelope of virions (Figure 11). The replication cycle is approximately 8-16 hours (Whitley *et al.*, 1998). Thus, transcription of viral DNA takes place in nucleus, while all viral proteins are synthesized in cytoplasm of the infected cell (Taylor *et al*., 2002).

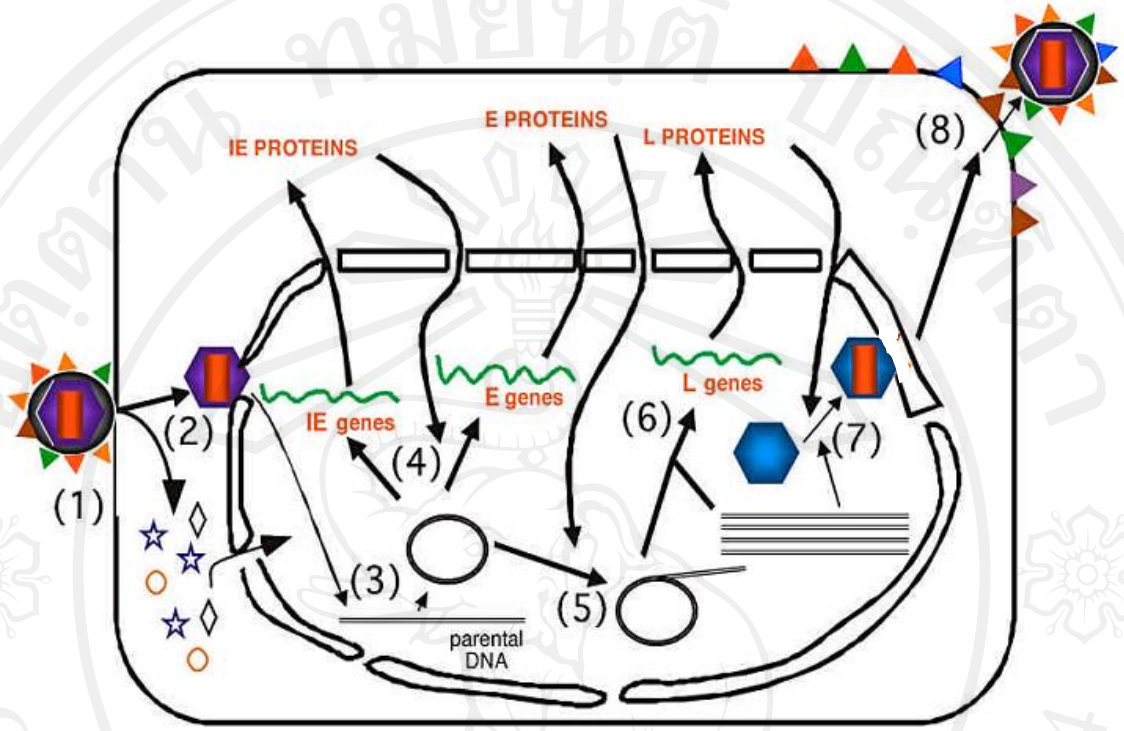

Figure 11 Replication of HSV cycle. The cycle of HSV infection are composed of (1) HSV particle binds to receptor on cell surface membrane and fusion (2) Viral nucleocapsid and tegument are released into cytoplasm of host cell and transported their nucleocapsid through nuclear pore (3) Viral DNA is released into nucleus (4) Transcription and translation of immediate early and early genes (5) Viral DNA synthesis (6) Transcription and translation of late genes (7) Encapsidation (8) Release of viral particle (Taylor *et al*., 2002).

After infection, the virus uses the intracellular components of host cell to express viral proteins for replication and other process. Two types of proteins; nonstructural and structural proteins are expressed (Taylor *et al*., 2002). More than 80 HSV proteins are expressed in a highly regulated cascade fashion in a number of coordinately expressed groups of gene products that play roles in regulation of viral gene expression. HSV-1 and HSV-2 encode at least 84 different polypeptides and 45 genes are necessary for functions such as viral attachment, DNA repair and immune response for viral infection (Whitley *et al*., 1998).

Furthermore, at least six viral proteins have important roles in expression of viral genes and have efficiently to mobilize cellular proteins for synthesis of viral DNA and proteins. Anti-tumor necrosis factor (αTNF) is a transactivator and essential structural protein of virion. Moreover, the infected cell protein 4 (ICP4) is the large complex component and it can directly bind to both high and low affinity sites at transcription initiation site. Therefore, ICP4 activates early and late genes during viral lytic cycle while IE genes are repressed (Compel and Deluca, 2003). ICP0 is promiscuous transactivators of HSV genes that is expressed in both nucleus and cytoplasm and has important functions such as stabilization of cell cycle and also regulation of protein synthesis and proteolysis (Hancock *et al*., 2009). Another, α protein, ICP22 is repressor of cellular cycle and promotes viral gene expression. Regulatory proteins ICP0, ICP4, and ICP22 are phosphorylated by cellular kinases (cell cyclin kinase, cdc2) and viral protein kinases (Us3 and U_L13) (Guo *et al*., 2010; Whitley and Roizman, 2001).

ICP27 is a transporter or late RNA from nucleus to cytoplasm and regulates the expression of late proteins. In addition, ICP27 is able to control the posttranslational processing of RNA (Figure 12).

ICP47 is another multifunctional protein which blocks RNA splicing in early infection (Figure 12). Tegument protein is activated in HSV replication. Therefore, two viral proteins, vhs and protein kinase are important for infection of viral disease. Vhs is the product of gene U_L41, which activates RNA activity for degradation of all mRNA (Amen and Griffiths, 2011).

In addition, RNA polymerase II of host cell is responsible for synthesis of all viral mRNAs. Viral proteins are necessary for initiation and enhancement of transcription of certain genes. Therefore, viral genome also contains signals for processing of newly synthesized genomes for packaging into pre-formed capsids**.** Transcription of viral genome is controlled by cellular DNA-dependent RNA polymerases and is regulated by viral-encoded and cellular nuclear factors. Lytic, persistent or latent infection is controlled under these factors. However, cells that promote latent infection restrict transcription to specific genes without genome replication (Taylor *et al*., 2002).

Latency-associated transcripts (LATs) are produced by specific region of genome during a latent infection, and detectable only while viral replication does not proceed. Early protein such as DNA-dependent polymerases is required to promote replication. Moreover, other early proteins inhibit production and initiate degradation of cellular messenger RNA and DNA. Thus, expression of early and late genes leads to cell death (Block and Hill, 1997; Kent *et al*., 2003).

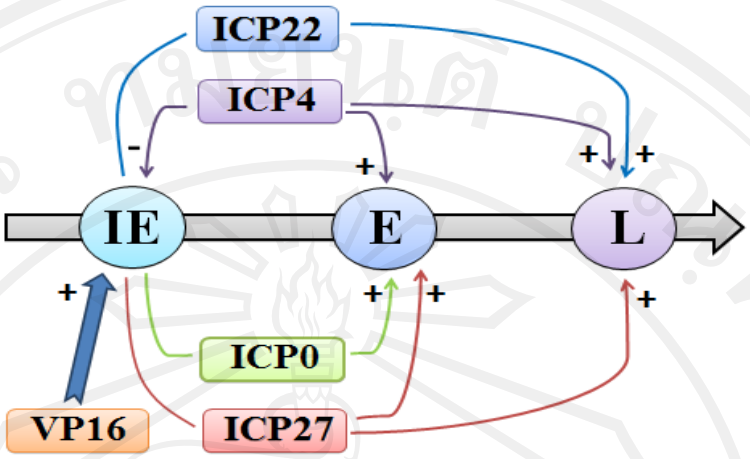

Figure 12 HSV gene expressions (modified from Simonato *et al*., 2000)

7. Virion assembly and release

Transcriptions of several early genes are needed to produce mature virus and their proteins are produced following DNA replication. Capsids are produced in nucleus and traverse at inner nuclear membrane, outer nuclear membrane and cytoplasmic membrane to release from the infected cell. Procapsids are precursors of mature capsid and formed from scaffolding that are necessary for initial DNA cleavage. They do not contain viral DNA, unstable and porous. Furthermore, procapsid had angular in shape that is lower than other type of capsid. C-capsids are found in mature infectious virions, which compose of packaged viral genomes. The scaffolding protein consists of a protease that can cleave substrate during capsid assembly. B-capsids do not contain viral DNA as well as A-capsid but they contain cleaved scaffolding protein. Assembly of viral capsid is occurred in nucleus, which requires synthesis of late proteins. However, VP5, VP23 and VP26 are capsid proteins, which lack nuclear localization sequences (NLS). Thus, these proteins form complex with NLS-containing protein in cytoplasm in order to transport into nucleus.

Encapsidation occurs in nucleus after newly viral DNA is synthesized by cleavage of HSV DNA concatemers into unit-length monomers molecules, which requires several viral gene including the U_L6, U_L15, U_L25, U_L28, U_L32, U_L33, U_L36 and U_L37. Then, emply capsid shells are loaded and packaged with viral DNA by a process that simultaneously resolves concatemers into the virions (Taylor *et al*., 2002; Whitley *et al.,* 1998). In addition, polyamines are facilitated to the encapsidation process but histone is not required (Roizman *et al*., 2007).

After encapsidation of full-length viral genomic DNA molecules, nucleocapsids attach to the nuclear surface of inner nuclear membrane and rapidly enveloped by budding and released into the space between the inner and outer nuclear membranes that contains the viral glycoproteins. All glycoproteins are synthesized and then receive the high-mannose sugar chains at the endoplasmic reticulum and diffuse to the nuclear membrane (Whitley *et al*., 1998). Interactions between capsid and tegument proteins and between tegument proteins and viral glycoproteins in the inner nuclear membrane promote this budding process. This early process takes place in nucleus and capsid is surrounded by the primary tegument protein. After egress start, disruption of nuclear lamina involving HSV proteins U_L43 and U_L34 phosphorylated membrane proteins has been inserted. Capsids are coated with tegument proteins and bind with inner nuclear membrane containing U_L31/U_L34 and HSV membrane glycoproteins gB, gD, gH/gL and gM, and are bud into perinuclear space (Whitley *et al*., 1998).

At perinuclear, the primary enveloped capsid buds after glycoproteins are processed via outer nuclear membrane where the primary envelope is de-enveloped, the nucleocapsids are delivered into the cytoplasm by transferring in a vesicle to the

Golgi apparatus via areas where viral proteins are concentrated and released into cytoplasm (Farnsworth and Johnson, 2006). Here, virions become encased within transport vesicles and are transported through vesicles that are formed by fragmentation and dispersion of Golgi stacks to the extracellular space. These delivers enveloped virions into cytoplasmic vesicles and subsequently move into the cell surface. Secondary envelopment occurs as herpesvirus tegument-coated capsids bind onto viral glycoprotein-enriched regions of the Golgi apparatus, trans-Golgi network (TGN), or endosomes, which is known as secondary envelopment and its basolateral cell surfaces promote virus spread to other cells (Whitley and Roizman, 2001; Whitley *et al*., 1998). Then, enveloped virions within membrane vesicles are transported to cell surfaces, where there are fusion between vesicle and plasma membrane. The virus is released by budding pathway and virus is able to spread from cell to cell that is protected from host response (Figure 13) (Johnson *et al*., 2011). This process takes about 18 hours (Whitley *et al.,* 1998).

Therefore, the virus that is released from one cell can infect another cell by transportation across neighboring cells. Rapid cell-to-cell spread within the epithelium and HSV directly spread from one cell to another cell through the extracellular space, which involves a set of viral glycoproteins; gE and gI. Both gE and gI mediate HSV transfer across cell junctions by interacting with cell junction components (Taylor *et al*., 2002; Turner *et al*., 1998). However, virus induces cell fusion at approximately 5 hours post infection at 37ºC. At that time infectious virions are produced and released out of infected cells (Melancon *et al*., 2005).

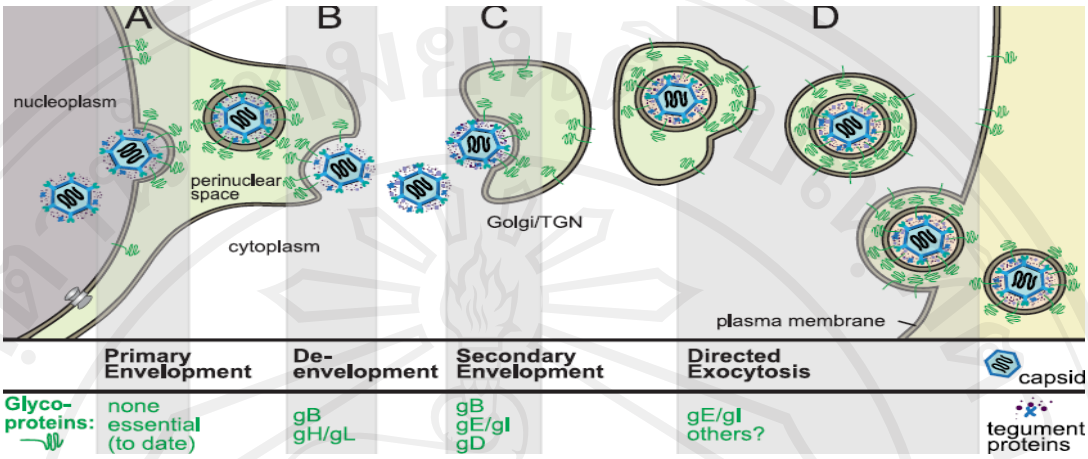

Figure 13 HSV release from infected cell (Johnson *et al*., 2011)

8. Pathogenesis and Immunity

HSV infections are a major morbidity due to virus is neurotropic and establishes latency in neurons of dorsal root ganglia and autonomic nervous system in primary sensory neurons (Lachmann, 2003; Whitley and Roizman, 2001).

Pathogenesis of HSV disease depends on types of HSV. Both types of HSV can infect human and other animals but only human are present the symptom of disease. Clinical manifestation of HSV infectious disease exhibits different severity, which depends on the port of entry, immune status, and also depends on primary or recurrent infection in hosts (Khan *et al*., 2005).

HSV enters susceptible individual by personal contact between excreting HSV through mucosal skin surfaces. Viral replication is at the site of infection and an intact virion is transported retrograde by neurons to the dorsal root ganglia. Thus, latent infection is established in the innervating neurons and hid from immune response. After viral infection, parabasal and intermediate cells of epithelium swell with condensed chromatin in nucleus. Then nuclear degeneration and cell membrane

breakage are detected in the cell. Therefore, at least 48-72 hours after viral infection, cytopathogenic effect (CPE) are produced in infected cells (Whitley and Roizman, 2001).

Generally, HSV-1 infection is mainly associated in predominates cause of orofacial infections. The virus can infect and produce skin lesions around face, mouth, lips, which is commonly called cold sores or fever blisters. HSV-1 replicates in mucosal epithelia cells in primary infection and results in either symptomatic disease at the site of viral entry or asymptomatic infection. During infection, the virus becomes latent in their local sensory ganglion, trigeminal ganglia and reactivation of virus causes the lesions at or near site of viral entry into the body. HSV-2 usually associates with infection of genital areas and can be latent in lumbosacral ganglia and spread through sexual contract (Riley, 1998).

Periodically reactivating of latent virus causes symptomatic lesions or undergo asymptomatic. Viral shedding may be systemic symptoms. Frequency of HSV-2 recurrence is greater than HSV-1 when infection involves the genital area. HSV-1 can cause serious diseases such as cornea keratitis and encephalitis. Neuoinvasiveness of HSV infection is invading the brain causing neurotoxicity from cytoskeletal disruption, and senescence of cells. Therefore, HSV can cause lytic infection of most cells, persistent infection of lymphocytes and macrophage, and latent infection in neurons.

These pathogenesis and symptoms of HSV-1 and HSV-2 infection are similar but the differences between two types of viruses including growth characteristics, antigenicity and their diseases. Generally, HSV-1 is usually associated with infections above the waist such as lips, mouth and throat while HSV-2

occurs at genital, cervix, vulva, vagina, penis and may occur on another area such as legs and buttocks. Both of oral and genital herpatic may be associated by red, itchy, painful, fever, headache and muscle ache.

First episode of disease tends to be more severe than recurrences. Initial lesion appears as the clear vesicle containing infectious virus that are formed on erythematous base and progresses to pustule lesions, ulcers, and crusted lesions. Therefore, reddened area is present and gives rise to a macula, which crusts to form a papula and fluid in the blister is fully of virus. In addition, the symptomatic oropharyngeal disease is characterized by lesions of the buccal and gingival mucosa, and fever.

Intraoral ulceration mostly occurs during primary infections whereas lesions on lip suggest recurrent infection. The orolabial lesions are preceded by pain, burning, tingling, or the vermillion border of the lip. Vesicles usually occur and persist for 48 hours then pustules or ulcers and crusts are formed within 72-96 hours. The lesions are completely healed after 8-10 days. Primary genital herpes also appears as macules and papules, follows by vesicles, pustules, and ulcers. The symptoms of an infection include burning or tingling sensations, following by multiple painful vesicles at the site of infection (Whitley *et al*., 1998). The complications after HSV infection include paraesthesias and dysaesthesias of the legs and perineum. Dysuria, localized inguinal adenopathy, and malaise are also formed (Whitley and Roizman, 2001). Therefore, cytolysis occurs from induction of multinucleated giant cells with condensed chromatin in the nuclei of the cells, followed by degeneration of the cellular nuclei and macromolecule within the epithelium. Vesicular fluid contains large amount of virus, cellular debris,

inflammatory cells and multinucleated giant cells. The vesicular fluid becomes pustular with the recruitment of inflammatory cells and subsequent formation of scabs. Thus, vesicles are replaced by shallow ulcers. Scarring is uncommon when mucous membranes are involved. In addition, present of virus indicates by formation of syncytia or Cowdry type A inclusion bodies in nucleus. Cowdry type A acidophilic intranuclear inclusion bodies are produced after changes in the nuclear structure and followed by glomerate of chromatin. However, presence of clinical symptoms is variable since tissue damage and inflammatory response are caused by a combination of viral pathology and host immune response (Fatahzadeh and Schwartz, 2007).

Primary episode of HSV-2 expresses multiple painful vesicles in clusters on a surface and may be associated with puritis, dysuria, vaginal discharge, and tender regional adenopathy. Moreover, many women have experience on fever, malaise, and myalgia 1 to 2 days before the appearance of lesions and the lesions may last 4-5 days before crusting but skin may not re-epithelialize for almost 10 days. Whereas, non-genital lesions may arise on mouth, buttock, legs, fingers, and eyes. On the other hand, symptomatic recurrent of HSV episodes are characterized by a prodromal followed by painful, vesicular lesions and fewer. Moreover, difference in recurrence rate depends on HSV types, which recurrence of HSV-1 is less frequent than HSV-2.

Cell characteristics of primary and recurrent infection of both types of HSV are associated with viral-mediated cell death and inflammatory response. The first line of cellular defense of HSV infection is innate immune system, which composes of main function to limit further infection and initiate cellular and humoral-mediated immune system (Chan *et al*., 2011). After infection, the reactivity of lymphocyte

blastogenesis develops within 4-6 weeks and sometimes as early as 2 weeks. Host defenses against HSV infections involve multiple immune cells such as macrophages, natural killer cells (NK cell), different T-cell sub-populations, and antibodies that mediate neutralization and/or antibody-dependent cell mediated cytotoxicity and cytokine response (Marques and Straus, 2000). The humoral immune response is important to reduce the viral titers during primary infection but does not prevent either recurrences or exogenous reinfection. Cellular immune response is the main factor in determining both the severity and the rate of primary infection as well as recurrence of HSV. However, the severity of HSV infection is related to the level of cellular immune response of host cell (Marques and Straus, 2000; Whitley *et al*., 1998).

Cell mediated immunity is important to control HSV infection. During primary infection, interferon (IFN), cytotoxic T cells and macrophages are essential for controlling HSV infections and are able to kill infected cells by IFN, which is important in limiting the initial infection. Moreover, NK cell are acted to limit and resolve the progression of the infection. Macrophages are activated by delayed-type hypersensitivity and cytotoxic killer T-cell responses. However, the immunopathology are caused by cell-medicated and inflammatory response of the disease symptoms. If functional of cell mediated immunity inactive, HSV infection may spread to the necessary organs and the brain (Whitley and Roizman, 2001).

During primary infection, immunoglobulin M (IgM) antibodies appear transiently followed by IgG and IgA, which persist for long periods. The humoral immune responses limit viral spread by neutralization of surface glycoprotein of extracellular virus and direct coating itself with IgG via Fc receptors and complement

receptors. However, virus can avoid neutralization of antibody by latent infection in neuron. Moreover, virion and virus infected cell may express antibody and complement receptor that can bind to antibody and complement and weaken these humoral defenses. Virus entry to axonal rapidly before NK cell or the IFN response occurs. Therefore, reactivation and reinfection may occur even in the presence of antibodies (Whitley and Roizman, 2001).

Programmed cell death is also the host defense to response viral protein or host immune system. HSV can block programmed cell death by its own or by host proteins. At least 3 proteins, U_S3, gD, and gJ can block programmed cell death by specific cell injury. HSV can make complementary RNA that can anneal and activate host protein kinase R (Whitley and Roizman, 2001).

Once virus enters trigeminal ganglion (TG), neurons are destroyed and are probably responsible for stimulation an inflammatory response. LAT expression is measured in neurons infected with latent virus. Therefore, prolonged inflammatory reaction in the TG is observed. This inflammation persists well beyond the time when viral replication or viral antigens are detectable and the prominent cell is the cluster of differentiation 8 (CD8)+ T lymphocyte. In addition, CD8+ T cell can block HSV-1 reactivation from latency in sensory neurons. Several cytokines and chemokine with IFN γ and TNF are produced from the inflammatory cells in the TG (Khanna *et al*., 2004). However, the virus can be reactivated despite the presence of antibody (Figure 14).

However, HSVs have ability to evade the host defense by interaction of viral protein with cellular proteins. The interactions of viral and cellular proteins are involved in blocking host cell response. HSV α protein (ICP47) can block the

presentations of antigenic peptides by binding to the transporter protein to transporter associated with antigen processing 1 (TAP 1) or TAP2 and block transporting peptides to the endoplasmic reticulum. Moreover, HSV has a system for blocking host responses against infection because the cells degrade some of the newly synthesized viral proteins (Whitley and Roizman, 2001).

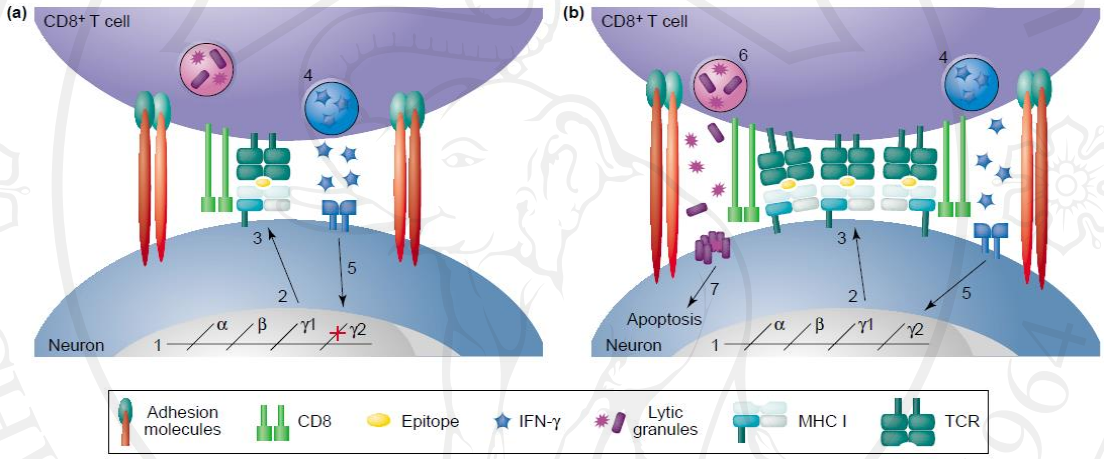

Figure 14 Immunology models of HSV-1 latency and reactivation (Khanna *et al*., 2004)

9. Transmission and disease of HSV

HSV infections are distributed worldwide and human is reservoir for transmission to susceptible individuals during close personal contact while animal vectors of human HSV infection have not been found.

HSV-1 and HSV-2 have different routes and involve different areas of the body and the two types of virus overlap in sign, symptom, epidemiology and clinical manifestations (Spear, 2004; Whitley *et al*., 1998). HSV-1 is more likely to reactivate frequently from oral sites and HSV-2 is more likely to reactivate from genital sites

(Spear, 2004). Therefore, recurrent infection is varies between men and women by 2.7 and 1.9 times per 100 days, respectively. In addition, women with initial genital herpes can shed the infection without symptoms which occur by 12% of primary HSV-1, 18% of primary HSV-2 and 23% of non-primary HSV-2 infection (Whitley and Roizman, 2001).

HSV-1 prevalence varies with age, race, geographic location, and socioeconomic status worldwide. The higher rate of seropositivity has been reported from less industrialized countries. Therefore, HSV-1 is the large proportion of population as a result of poor hygiene in underdeveloped countries that HSV-1 antibodies are found in more than 90% of children. The lowest rates of infection are found in children while the highest rates are in prostitutes and among them 80% are infected with HSV-2. Therefore, HSV-1 is primarily associated with oral, pharyngeal, facial, ocular, and central nervous system infections, which are transmitted by oral secretions and non-genital contract. Moreover, it can remain viable on the skin, clothing or plastic for short time and accommodates transmission through close nonsexual contact such as kissing on the cheeks or sharing common wares (Whitley and Roizman, 2001).

Genital herpes is very common and most affects adult people. It is important sexually transmitted disease (STD) caused by HSV-2 infection and the exception of a minority of cases caused by HSV-1 (Khan *et al*., 2005; Kriebs, 2008). Therefore, older age, female gender, race, poor socioeconomic status, low level of education, sexually transmitted disease, early age at first intercourse and a higher number of lifetime sexual partners lead to the risk of genital herpes infection in human (Fatahzadeh and Schwartz, 2007). Furthermore, HSV-2 is seroepidemiologically

associated with human cervical cancer, possibly as a cofactor with human papillomavirus (Brugha *et al*., 1997; Gupta *et al*., 2007)**.**

Vertical transmission of neonate from an infected mother to her baby usually occurs during vaginal delivery pregnancy. Maternal genital infection during the time of delivery, the quantity and quality of maternal antibodies, duration of ruptured membranes in the presence of active infection and the use of fetal scalp monitor during delivery may affect neonate infection (Anzivino *et al*., 2009; Brugha *et al*., 1997; Piret and Boivin, 2011; Sacks *et al*., 2004).

Oral herpes

Oral herpes is the most common manifestation, which is caused by HSV-1 infection. The lesions are generally occurred near oral mucosa on lips and facial area and it spreads to all parts of the mouth.

The symptom of oral herpes infection appears as a small group of clear vesicles, which is commonly called fever blisters or cold sores. Itching, pain, enlarged submandibular lymph node, sore throat, malaise and rapidly ulcerative lesions distribute throughout the mouth involving the hard palate, gums, chest, pharynx, gingivae, buccal mucosa, lip and the top of tongue. In addition, it is also possible that HSV can be reactivated from the trigeminal ganglia and results in the recurrent HSV infected lesions at the same location inside the mouth. However, the symptoms of recurrent episode are less severe and more localized than primary episode (Lachmann, 2003).

Herpetic gingivostomatitis

Gingivostomatitis is common symptom of primary HSV-1 and HSV-2 infection in children. Therefore, children are almost infected by HSV-1, whereas young adults may be infected with HSV-1 or HSV-2.

The symptoms of herpetic gingivostomatitis include painful vesicular lesions, fever and submandibular lymphadenopathy. The ulcerative lesions affect skin and mucous membranes. However, majority of infections are subclinical (Marques and Straus, 2000).

Herpes pharyngitis

Clinical symptoms of herpes pharyngitis are ranged from mild to severe pharyngitis. Herpes pharyngitis occurs with sore throats. The patients with HSV pharyngitis exhibit ulcerative lesions, vesicles at posterior pharynx with exudative lesions following the illness with the fever, malaise, and myalgia (Marques and Straus, 2000).

Herpes stomatitis

Herpes stomatitis is infection of oral cavity that commonly causes blisters, fever, itching, trauma, ulcerative lesions and inflammation. Severe HSV stomatitis is similar to a primary gingivostomatis symptom and may occur in immunosuppressed patients (Pithon and Andrade, 2010).

Herpetic keratitis and HSV keratoconjunctivitis

Herpetic keratitis caused by HSV-1 infection occurs in cornea of eye and this infection almost limits to one eye. The symptoms are an acute onset of pain, watery discharge, itching, blurred vision, lid swelling, and conjunctiva of eye (Spear, 2004). Thus, primary infection causes an acute corneal damage, and recurrence or

chronic epithelial keratitis leads to involvement of deeper layer of cornea, which causes severe dendritic ulcers, permanent corneal scarring, corneal damage and major cause of corneal blindness (Lachmann, 2003).

HSV keratoconjunctivitis is ocular infection with HSV-1 infection of eye and associated with unilateral or bilateral conjunctivitis. For primary infection the bilateral keratoconjunctivitis manifests with a pain, watery discharge, itching, blurred vision, lid swelling, photophobia, tearing, and eyelid edema. While, recurrent disease is caused by viral shedding from reactivation of virus in trigeminal ganglia, this is unilateral and causes dendritic ulcers leading to corneal scarring. Therefore, the superficial ocular infections involve the eyelids, conjunctiva or corneal surface and lead to serious disease causing vision loss. In addition, retinal necrosis is also associated with HSV-1 infection (Marques and Straus, 2000; Whitley *et al*., 1998).

Herpetic whitlow

Herpetic whitlow disease is infection of finger by contact with HSV from body secretions. The virus enters the body and establishes infection through abrasions or through small wounds on the hands or wrists skin. HSV-2 whitlow is caused by contact of HSV-2 from genitals to hands. Lesions are present at fingertip and can be pustule and very painful. In addition, fever and local lymphadenopathy are commonly seen (Marques and Straus, 2000; Spear, 2004).

Eczema herpeticum

Eczema herpeticum or Kaposi varicelliform shows clinical skin symptom that is present as active eczema, preexisting atopic dermatitis and small blisters. Infection can be spreaded along skin at the site of eczema lesions. It also has

potential to affect adrenal glands, liver, adrenals and other organs. This infection is rarely found but it is severe disease (Gupta *et al*., 2002).

Genital herpes

Genital herpes is life-long and commonly results from HSV-2 infection but about 10% of cases are from HSV-1 infection. This disease is public health concern due to its serious psychosocial morbidity and frequency recurrent painful genital lesions are occurred several times in a year (Khan *et al*., 2005; Marques and Straus, 2000). Genital herpes are transmitted by direct contact of recipient's mucous membranes or skin infected with sexual partner (Brugha *et al*., 1997; Khan *et al*., 2005; Spear, 2004).

The clinical apparent of genital herpes infection may be symptomatic or asymptomatic depending on multiple factors such as the site of infection and immune statue of host. Therefore, the diseases are often occurred more than asymptomatic infection and the diseases show as macules, papules and pustules with ulcers. In men, lesions have been found on glans or shaft of penis and sometimes in urethra whereas lesion may be seen on vulva, vagina, cervix, perianal region, or inner thigh in female patients. These painful lesions may be synchronous with a variety of systemic signs and symptoms including fever, malaise, headache, myalgia, inguinal adenitis, dysuria, itching, vaginal and urethral discharge, tender inguinal lymphadenopathy and glandular inflammation of groin area. Secondary episodes of genital herpes result from viral reactivation from sacral ganglion. The infections are frequently less severe than first episode (Anzivino *et al*., 2009; Brugha *et al*., 1997; Marques and Straus, 2000).

Moreover, HSV proctitis can be found. It is inflammation lesion in lower rectum and anus area with pus, blood discharge, painful and irritation around infection area. Primary episode of this infection is longer in duration and more severe than recurrent genital HSV infection. Moreover, recurrent infection may be asymptomatic but the virus can be spreaded to other people (Whitley *et al*., 1998).

HSV meningitis and encephalitis

Neurovirulence of HSV infection is neuroinvasiveness of virus from peripheral sites and replication of virus in neuronal cells. Therefore, the virus can invade and replication in central nervous system (CNS) and also can be established latent infection (Whitley *et al*., 1998).

HSV meningitis is often a complication of primary genital HSV-2 infection. The patients with HSV meningitis have headache, fever, stiff neck and mild photophobia (Marques and Straus, 2000).

HSV encephalitis is usually associated with HSV-1 infection in brain. The disease generally shows acute onset of focal neurologic symptoms and fever (Lachmann, 2003; Marques and Straus, 2000). Pathology of this infection causes damage to temporal lobes of the brain and focal necrosis. The infection also gives rise to erythrocytes in the cerebrospinal fluid, seizures, focal neurological abnormalities, and other characteristics of viral encephalitis. However, the lesion is limited in one of temporal lobes (Sauerbrei *et al*., 2000). The most severe sporadic encephalitis causes high morbidity and mortality from CNS damage (Spear and Roizman, 1972). Herpes encephalitis can be demonstrated from the primary infection or secondary infection (Lachmann, 2003).

HSV infection in the neonate and newborn

HSV infection of neonate has a very high mortality, which results from infection with either HSV-1 or HSV 2. The infection is frequency occurred during passage of infant through genital birth canal of mother. The direct inoculation with infectious genital secretions is found in mother with genital ulcers (Lachmann, 2003). Neonatal herpes may occur in the absence of skin lesions. The infection of HSV in neonates during and after birth is localized around skin, eye and mouth. However, neonate has an underdeveloped immune system and virus can spread rapidly into many peripheral organs (Kimberlin, 2004).

10. Laboratory diagnosis

Clinical laboratory diagnoses of viral infections are detected and confirmed by several methods. Therefore, specific and sensitivities of the test depend upon the type of test and quality of specimen obtained. However, detection of diseases in asymptomatic individuals is still limited. Diagnosis procedures include viral antigen, viral DNA, infectious virus and viral antibodies detection.

Clinical specimens are taken from lesions by collecting the lesion fluid or cotton swab to the skin vesicles, eyes, mouth, cerebrospinal fluid (CSF), rectum, blood, stool, urine, throat, nasopharynx, and corneal lesion. All of clinical specimens are transferred into media and sent to virology laboratory (Whitley *et al*., 1998). Diagnosis HSV infection is also performed from biopsy, viral culture, antigen detection by enzyme immunoassay, immunofluorescence, detection of nucleic acid with PCR, microarray technology, and loop mediated isothermal amplication (LAMP) (Navaneethan *et al.,* 2010).

10.1 Cytology technique

This methodology is standard method for diagnosis HSV infectious specimen, which shows positive result in cell culture within 2-3 days after inoculation. HSV determination in cultured cells is obtained from scraping of skin vesicles called Tzank smear, which approximately 60% sensitive. Papaanicolaou (PaP) smear or biopsy specimen can also be used to demonstrate cytologic changes of tissue culture cells.

Detection of HSV infection in cell culture was observed by characteristic of cytopathological effects (CPEs), which are present of enlarged foci, syncytia "ballooning" cytoplasm and multinucleated giant cells formation. Moreover, Cowdry type A intranuclear inclusion bodies are also shown. However, cytology technique is less sensitive than detection by immunological methods (Brugha *et al*., 1997).

10.2 Serological assays

Detection of HSV-1 and 2 infections by serological assays are widely used for diagnostic a primary HSV infection and epidemiological studies. However, serological diagnostic of HSV infection can only determine the past exposure because the rise of antibody titers is not found in recurrent disease (Marques and Straus, 2000; Riley, 1998).

Most laboratories analyze subtype of viral isolates using highly sensitive and type-specific serological assays. Monoclonal anti-HSV antibodies are used in immunodot enzyme assays (IEA), fluorescent antibody assay, immunoperoxidase method, enzyme-linked immunoabsorbant assay (ELISA) and western immunoblot analysis (WBA) assays (Brugha *et al*., 1997; Riley, 1998; Wald *et al*., 2001).

10.3 Molecular method

Determination of HSV type is detected by molecular method using restriction endonuclease cleavage of HSV DNA and DNA hybridization using HSV type-specific DNA probe to detect different types of HSV and PCR technique. HSV DNA is amplified by using a common appropriate type-specific forward primer and reverse primers for HSV-1 and HSV-2 (Clark *et al*., 2011). This technique shows specific, sensitive biological technique and rapid detection more than viral culture. Therefore, PCR is recognized as the reference standard method to detect herpes simplex encephalitis caused by HSV infection (Madhavan *et al*., 1999; Riley, 1998; Whitley *et al*., 1998).

11. Prevention and treatment HSV infection

Direct contact with herpes blister on mucocutaneous lesion from patients should be avoided to reduce the risk of infection because HSV can spread by close contact and during sexual intercourse. However, symptoms of some HSV infected patient are unapparent so virus can transmit without prevention. Additionally, people who work in hospital must be careful when handling potentially infected tissue or fluids and should wear the gloves to prevent infection of the fingers and clean by washing soap (Riley, 1998).

HSV is an enveloped virus so it is easily inactivated by dryness and detergents (Brugha *et al*., 1997; Danaher *et al*., 2011). Nowadays, HSV infections are generally managed by potential antiviral drugs as specific inhibitors to virus at various stages in HSV replication cycle by inhibition of virus nucleic acid or protein synthesis. Furthermore, new anti-herpes drugs, which have alternative

pathways and different inhibitors on HSV activity, are also searched for potentially valuable target against HSV infections disease (Greco *et al*., 2007). Thus, the effective antiviral drug therapy for treatment HSV infection must selectively target to specific virus particle or viral replication cycle inside host cells without affecting on host cells metabolism. These effective drugs will reduce health care costs, shorten herpes disease episodes, shorten time to heal of HSV disease, and reduce recurrences of herpes and transmission. Additionally, antiviral agents that used in primary infection should able to reduce probability of patient suffering recurrent episodes (Lachmann, 2003; Jassim and Naji, 2003).

There are three different classes of anti-HSV drugs have been used. First, nucleoside analogs that acts as inhibitor of thymidine kinase (TK) activity in infected cell. Second, DNA polymerase inhibitors that acts as inhibitor of DNA polymerase activity of viral DNA polymerase. Third, helicase-primese inhibitors those are able to inhibit helicase and primase activity and also viral DNA synthesis. Although, several effective anti-HSV drugs are available for treatment HSV infection but the disease is not completely cured because latency establishment from virus since viral genomes in ganglia are not affected by drugs and most patients have recurrent HSV infection (Dobson *et al*., 1998; Lachmann, 2003; Whitley and Roizman, 2001). However, anti-HSV agents have been used to reduce clinical and subclinical rate of infection and long-term therapy with these agents may result in emergence of drug resistant virus (Lückemeyer *et al*., 2012).

Anti herpes simplex virus agents that have been approved for treatment of HSV infection are as follows

11.1 Acyclovir

Acyclovir (ACV) (9-[2-hydroxyethoxymethyl] guanine), is synthetic acyclic purine-nucleoside analogue. It has been known as effective and safety antiviral drug, and used for long-term prophylaxis with low side effects. It reduces clinical establishment of acute or latent infection severity in variety of clinical situations by reduction of the duration of pain, complications and viral reactivation (Dobson *et al*., 1998; Marques and Straus, 2000).

Therefore, ACV is prototype of antiviral agent that can be converted to its active form after phosphorylation by a viral encoded TK enzyme, which has 100-fold selective affinity greater than cellular polymerase. Then, ACV-monophosphate is subsequently phosphorylated by host cell enzymes to ACV-triphosphate. Thus, ACV-triphosphate becomes active only in infected cells. These incorporated ACV-triphosphate prevents chain elongation and terminates viral DNA synthesis lead to inhibit viral DNA polymerase and viral replication because of lacking a hydroxyl group in the 3′ position (Figure 15) (Lachmann; 2003; Marques and Straus, 2000; Piret and Boivin, 2011).

However, resistance strains may be occurred in those people that had long-term ACV prophylaxis. Predominantly of ACV resistance strains (95 %) result from generation at least one of base mutation. Thus, defective HSV mutants or deficient in TK are unable to phosphorylate acyclovir (Lachmann, 2003; Marques and Straus, 2000).

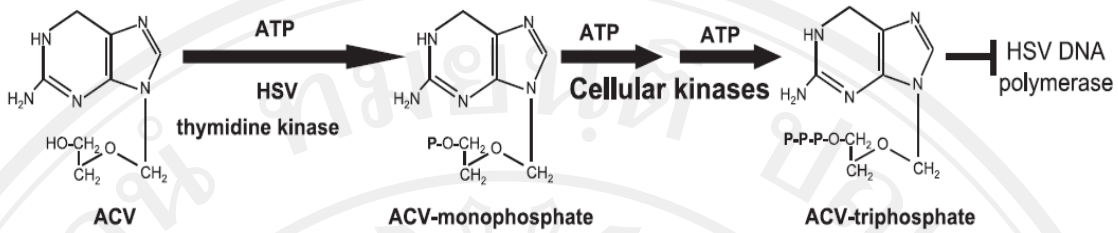

Figure 15 Mechanism of ACV inhibitor in HSV treatment (Griffiths, 2011)

11.2 Famciclovir

Famciclovir is a diacetyl ester prodrug analog compound. It is well absorbed in oral cavity. It is another guanosine analogue and able to convert rapidly by phosphorylation to its active antiviral metabolite, penciclovir triphosphate (Faro, 1998; Saltzman *et al*., 1994).

11.3 Valacyclovir

Valacyclovir is the L-valyl ester oral prodrug of ACV that shows similar viral inhibitory activity as ACV. The drug is completely absorbed in oral cavity, rapidly hydrolyzed and converted to acyclovir in intestinal wall and liver. Valacyclovir has effective affinity to viral TK enzyme. It can reduce subclinical and HSV shedding (Marques and Straus, 2000; Riley, 1998; Whitley *et al*., 1998).

11.4 Penciclovir

Penciclovir (9-(4-hydroxy-3-hydroxymethylbut-1-yl) guanine) is an acyclic guanine nucleoside analogue. A spectrum of activity and mode of action mechanism is similar to ACV. Penciclovir is phosphorylated and converted into penciclovir triphosphate. Viral DNA synthesis and replication are inhibited and chain elongation is limited (Piret and Boivin, 2011).

11.5 Cidofovir

Cidofovir (1 - [(S) - 3 - hydroxy – 2 - (phosphonomethoxy) propyl] cytosine dehydrate) is acyclic nucleotide phosphonate analog with broad-spectrum against HSV infection activity. Cidofovir is phosphorylated by cellular kinases to cidofovirtriphosphate to obtain active molecule and acts as competitive inhibitor of viral DNA polymerase (Lachmann, 2003; Marques and Straus, 2000).

11.6 Foscarnet

Foscarnet (trisodium phosphonoformate) is an organic analogue, which acts as viral DNA synthesis inhibitor. ACV-resistant infections strain can be treated by forcarnet, which is prevent the cleavage of pyrophosphate from deoxynucleotide triphosphates and the action is not requite phosphorylation. However, foscarnet is more toxic than ACV (Marques and Straus, 2000).

Mechanism and pathways of several antiherpes drugs are summarized in Figure 16.

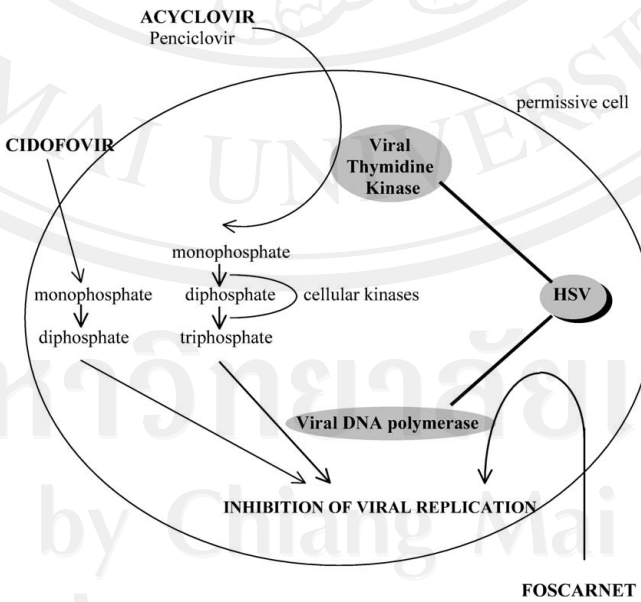

Figure 16 Mechanism of antiviral against herpes simplex virus infection (Morfin and Thouvenot, 2003)

12. Antiviral agent from medicinal plants

Although, various effective synthetic drugs have been used for treatment of HSV infections but the drugs are very expensive and some patients may not be able to afford the cost of long-term treatment and the possibility of their toxicity and their side effects (Khan *et al*., 2005; Tragoolpua and Jatisatienr, 2007; Yoosook *et al*., 1999). Moreover, problem of viral resistance strain has been increased and led scientists to discover new antiviral agent against HSV infection, which has different mechanisms of action and increased bioavailability (Spear and Roizman, 1972). Consequently, new antiviral agent with effective and inexpensive antiviral therapy that exhibit specific inhibition to viral functions, and non-toxic to host cells are needed (Allahverdiyev *et al*., 2004; Armaka *et al*., 1999; Mandal *et al*., 2008).

Medicinal plants are great important source for primary health care of individuals and communities. About 60-80% of the world populations continue to use medicinal plant in folk medicine as alternative way to treat and prevent various infectious and non-infectious diseases in human and animals such as diabetes, kidney and urinary bladder disturbances, intestinal infections and microbial infections (Igbinosa *et al*., 2009; Ogbonnia *et al*., 2008).

Ethnopharmacological knowledge leads the scientists and pharmacists develop new bioactive compounds of antiviral agents from medicinal plants as a potential source of substances with significant pharmacological and biological active compounds (Jassim and Naji, 2003; Ogbonnia *et al*., 2008; Serkedjieva and Ivancheva, 1999; Tiwari *et al*., 2011).

Natural products from medicinal plants are sources of natural compounds growing in various areas, which provided varieties of pharmacological properties and

herbal remedies as great alternative sources, and have the advantage, easily available, inexpensive, safe, and efficient but have little or no side effects (Akanitapichat *et al*., 2006; Ananil *et al.*, 2000; Armaka *et al*., 1999; Bukke *et al*., 2011; Nirmala and Selvaraj, 2011; Tolo *et al*., 2006).

Recently, the utility of medicinal plant is increasing globally with intense scientific investigation on their biological activities in order to develop new drugs (Armaka *et al*., 1999; Jassim and Naji, 2003; Mathur *et al*., 2011; Yadav and Agarwala, 2011). The most important variety of bioactive active compounds including alkaloids, tannins, terpenoids, flavonoids, phenolic compound, lignans, sulphides, polyphenolics, coumarins, saponins and anthraquinones (Anago *et al*., 2011; Edeoga *et al*., 2005). Therefore, medicinal value of these plants has many chemical substances that produce a multiple physiological and pharmacological action on the human body. Combination of two or more plant products may contain active constituents with multiple physiological activities and could be used in treating various diseases (Utsunomiya *et al*., 2008).

Extraction method of medicinal plants

Extraction procedures are necessary for laboratory to obtain therapeutic and biochemical active compounds from plant materials using suitable solvents. In extraction procedure, active constituents should be obtained without degradation. Extraction procedures should target on active constituents and not destroy sample during extraction. Different solvent system gives varieties of bioactive compound from plant material. However, various solvent that is suitable for extraction was considered (Table 2).

General methods for extraction of chemical compound in medicinal plant are sonification, heating under reflux, continuous extraction by Soxhlet's apparatus and other methods were used (Sasidharan *et al*., 2011).

Maceration is widely used. The medicinal plants are macerated in suitable ratio of solvent in closed container. The mixture is then kept at room temperature for 3 days with frequent stirring and repeated twice with fresh solvent. The extracts are combined and filtered. This method is suitable for initial and bulk extraction, which the extract is not degraded from heat. Medicinal plant containing thermolabile compounds can use maceration.

Continuous extraction by Soxhlet's apparatus is performed by placed the sample in thimble. Suitable solvent is added and then heated. The advantage of this procedure is continuous process and save the time.

Ultrasound-assisted solvent extraction is modified from maceration techniques where the plant is placed in ultrasound bath. Ultrasounds with frequencies ranging from 20 kHz to 2,000 kHz are induced and lead to the breakdown of plant components and increase the solubilization. However, it is used for initial extraction with small amount.

Table 2 Solvents used for active component extraction (Cowan, 1999)

Water	Ethanol	Methanol	Chloroform	Dichloromethanol	Ether	Acetone
Anthocyanins	Tannins	Anthocynanins	Terpenoids	Terpenoids	Alkaloids	Phenol
Starches	Polyphenols	Terpenoids	Flavonoids		Terpenoids	Flavonols
Tannins	Polyacetylenes	Saponins			Coumarins	
Saponins	Flavonoid	Tannins			Fatty acids	
Terpenoids	Terpenoids	Quassinoids				
Polypeptides	Sterols	Lactones				
Lectins	Alkaloids	Flavones				
		Phenones				
		Polyphenols				

13. Phytochemicals

Phytochemicals are naturally large group of plant derivative compounds. The name “Phyto” comes from ancient Greek word, which means plant. There are a variety of phytochemicals that have been found in medicinal plant and derivative. Phytochemicals have different biological activity properties (Allahverdiyev *et al*., 2004; Kumar *et al*., 2009; Raja and Sama, 2012). The phytochemicals found in plant and herbal sources have complementary, overlapping mechanisms of action against variety of disease (Jassim and Naji, 2003). Phytochemicals can be grouped as follows.

13.1 Flavonoids

Flavonoid is the large group of phytochemicals, which are polyphenolic compounds. It have been recognized that phytochemicals have variety potential biological functions and can inhibit human disease with anti-bacteria, anti-virus, anti-allergic, anti-platelet, anti-inflammatory, anti-tumor and anti-oxidant activities (James *et al*., 2009; Rispail *et al*., 2005; Xu *et al*., 2007).

13.2 Alkaloids

Alkaloids generally contain nitrogen-bearing molecule as pharmacologically active compounds. There are different types of alkaloids such as tropane alkaloids, sanguinarine, berberine and reserpine. Moreover, pharmaceutical of alkaloids have been reported such as anti-spasmodic and anti-bacterial activities (Yadav and Agarwala, 2011).

13.3 Saponins

Saponins are heterogeneous group of natural products found in many medicinal plants. There are two types of saponins; triterpenoid and steroidal

saponins. These compounds show various spectrums of biological and pharmacological activities such as anti-inflammation, anti-microbial and anti-viral activities (Yadav and Agarwala, 2011).

13.4 Tannin

Tannins are water-soluble phenolic compounds produced by all plants. It is a health-promoting compound and uses as anti-carcinogenic, anti-mutagenic, anti-microbial, anti-oxidant and anti-radiation activities (Amarowic *et al*., 2009; Karamać *et al*., 2007).

13.5 Anthraquinones

Anthraquinones are class of natural compounds found in many plant families, which are composed of various chemical compounds. These compounds show pharmaceutical bioactivity properties such as anti-microbial, anti-cancer and anti-oxidant activities (Dave and Ledwani, 2012; Locatelli *et al*., 2009; Mohanlall *et al*., 2011; Sakulpanich and Gritsanapan, 2009).

13.6 Cardiac glycosides

Cardiac glycosides are found in many medicinal plants, which contain digitoxin, digoxin and ditoxin. It has been recognized for treatment cardiac, congestive heart failure by helping to support its strength and rate of heart contraction.

13.7 Phenolic compounds

Phenolic compounds are one of the large and diverse groups of molecules that found in many different families of plant. It can be classified into non-soluble compounds such as condensed tannins, ligans and cell-wall bound hydrocinnamic acids. Soluble compounds are phenolic acids, phenylpropanoids, flavonoids and quinoes (Rispail *et al*., 2005). These compounds are impact on human health, which

show pharmaceutical properties such as anti-apoptosis, anti-aging, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activity (Yadav and Agarwala, 2011).

13.8 Coumarins

Coumarins are classified into benzopyrone family. It is a large class of compounds in plant. Various bioactive activities are shown on anti-microbial, anti-tumor, anti-malarial, anti-cancer, anti-inflammatory, anti-coagulant, anti-oxidant, anti-coagulant and anti-proliferative activities (Al-Haiza *et al*., 2005; Jain and Joshi, 2012; Mirunalini and Krishnaveni, 2011; Sahoo *et al*., 2012).

14. Antiviral activities of some medicinal plant extracts

The medicinal plants are sources of novel antiviral agent (Lipipun *et al*., 2003). Therefore, many researchers reported various species of medicinal plants that exerted efficacy against HSV infection. Various kinds of medicinal plants has been investigated on anti-HSV activity such as anti-HSV type 1 activity of the crude aqueous extracts from shoots of *Helichrysum aurenitens* (Meyer *et al*., 1996). Moreover, *Hypericum mysorense, H. hookerianum, Eucalyptus globulus, Psidium incanum, Phyllanthus niruri, Limonium brasiliense* extract also has been shown the potent inhibited HSV-1 infection (Davood *et al*., 2012; Faral-Tello *et al*., 2012; Vijayan *et al*., 2004). Anti-HSV activity of *Geranium sanguineum* Linn (Serkedjieva and Ivancheva, 1998) and *Rhus javanica* was determined (Nakano *et al*., 1998). Ethanolic extract of *Annona muricata*, aqueous extract of *Petunia nyctaginiflora* and extract of *Nerium indicum* were found to inhibit HSV-1 (Padma *et al*., 1998;

Rajbhandari *et al*., 2001). Moreover, lupenol, pure compound isolated from *Carissa edulis* showed strong anti-HSV-1 activity (Davood *et al*., 2012). Combination of *Geum japonium, Rhus javanica, Syzygium aromaticum and Terminalia chebula* extract and ACV were also demonstrated their efficacy on anti-HSV-1 activity (Kurokawa *et al.,* 1999) as well as combination of *Centella asiatica* and *Maclura cochinchinesis* extract also showed inhibitory effect on HSV infection (Yoosook *et al.,* 2000).

Flavonoid isolated from *Morus alba* extract could inhibit HSV-1 infection (Du *et al.,* 2003). Pandarin compound isolated from *Pandanus amaryllifolius* also showed potent inhibit HSV-1 infection (Ooi *et al*., 2004). Anti-HSV-1 activity of *Rhus havanica, Melia azedarach, Scoparia dulcis* and *Solanum torvum* were also demonstrated (Alche *et al.,* 2003; Arthan *et al.,* 2002; Hayashi *et al*., 1988; Kurokawa *et al.,* 1999).

Methanol extracts of *Barlaeria lupulina* and *Clinacanthus nutans* were active against HSV-2 (Yoosook *et al*., 1999). In addition, *Melissa officinalis, Myrica rubra, Euphorbia jolkini* and *Hyptis fasciculate* extract also showed potential effect against HSV-2 infection (Allahverdiyev *et al*., 2004; Cheng *et al*., 2003; Cheng *et al.,* 2004; Gomes *et al*., 2008).

Inhibition effect of HSV-1 and 2 were shown from a partially purified fraction derived form a dichloromethane-methanol extract of *Dunbaria bella* Prain (Akanitapichat *et al*., 2005). *Phyllanthus urinaria, Centella asiatica, Mangifera indica, Geum japonium, Plantago major* and *Euphorbia segetalis* extracts could inhibit HSV-1 and HSV-2 infection (Chiang *et al*., 2002; Khan *et al*., 2005; Madureira *et al*., 2003; Yang *et al*., 2005; Yoosook *et al*., 2000). Anti herpes simplex

virus 1 and 2 activity from crude water extract of *Carisra edulis* (Tolo *et al*., 2006) and the isolated compounds hyperbrasilol B, amentoflavone, and luteoforol from methanolic extract of *Hypericum connatum* Lam showed activity on HSV-1 (Fritz *et al*., 2007). In addition, fraction of *Mallotus peltatus* showed inhibit HSV-1 and HSV-2 infection (Bag *et al*., 2012) as well as *Clinacanthus nutans* and *C. siamernsis* extract (Kunsorn *et al*., 2013).

Other studies on antiviral activities of medicinal plants showed that aerial part of *R. nasutus* extract could inhibit influenza virus (Kernan *et al*., 1997). Moreover, antiviral activities of *Castanospermium austral, Stehania cepharantha, Drymaria diandra, Symplocos setchuensis* and *Ancistrocladus* korupensis were shown to inhibit HIV (Chattopadhyay and Naik, 2007). Moreover, *Stephania cepharantha* could inhibit HIV, SARS, HSV and CVB3 infection, while *Zanthoxylum chalybeum* could inhibit measles infection. In addition, *Eleutherococcus senticosus* and *Garcinia multiflora* could inhibit coronavirus whereas *Camellia sinensis* could inhibit rotavirus.

In addition, algae also demonstrated anti-HSV activity. Marine green alga, *Dunaliella promolecta* showed inhibitory effect on HSV infection (Ohta *et al*., 1998). Moreover, polysaccharide extracts from marine algae; *Undaria pinnatifida, Splachnidium rugosum, Gigartina atropurpurea* and *Plocamium cartilagineum* had antiviral efficacy against both types of HSV (Harden *et al*., 2009).

15. Plants used in this study and description

In Thailand, many medicinal plants have been widely used in folk medicine in primary health care to treat various diseases. The knowledge about folklore medicinal of plants is interesting to search for new antiviral agent. Thus in this study,

some Thai medicinal plants were selected base on pharmacological activity as follows.

15.1 *Andrographis paniculata* (Burm.f.) Wall. ex Nees

Family: *Acanthaceae*

Characteristics

Herbaceous plant is 30-110 cm in height. It is bitter in taste and branched herb, slender in shape and lobed flower. Lance-shaped leaves are 6.0-10.0 cm long and 3.5-5.0 cm wide. Stem is dark green, woody and glabrous (Figure 17).

Pharmaceutical properties

This plant is used for treatment of inflammation, fever, laryngitis, sore throat, diarrhea, thrombotic, stomachache, chronic infection, virus infection, malaria, skin eruptions, cancer and diabetes.

Bioactive constituents

Andrographolide, steroids, diterpenoids, alkaloids, andrograpanin, saponins, flavonoids, polyphenol, kalmeghnin were found as bioactive constituents (Benoy *et al*., 2012; Chao and Lin, 2010; Hosamani *et al*., 2011; Kumar *et al*., 2012).

Figure 17 *Andrographis paniculata* (Burm.f.) Wall. ex Nees

15.2 *Cissus quadrangularis* L.

Family: *Vitaceae*

Characteristics

This plant is climbing and dichotomously branched herb with 150 cm in height. Leaves are simple or lobed, cordate, broadly ovate or reniform, serrate, dentate and glabrous. Stem is quadrangular, buff color with greenish tinges, reddish-brown, no taste and smell. Flowers are small and greenish white (Figure 18).

Pharmaceutical properties

This plant is used for treatment of inflammation gonorrhea, tumors, hemorrhage, anemia, anorexia, asthma, abscess and syphilis.

Bioactive constituents

Carotenoids, triterpenoids, tannins, carotene, phenols, ketosteroids, flavonoids, calcium oxalate, linoleic acid, tannins, steroids, tetraterpenoids were found as bioactive constituents (Bum *et al.*, 2008; Kumar *et al*., 2012; Mishra *et al*., 2010; Shah, 2011).

Figure 18 *Cissus quadrangularis* L. (Shah, 2011)

15.3 *Coscinium fenestratum* (Gaertn.) Colebr.

Family: *Menispermaceae*

Characteristics

This plant is creeping and climbing vine, yellow wood and branchlets hoary. Leaf is single, alternate, 15-18 cm long and 13-15 cm wide, ovate, acuminated apex, lower blade white-green, rounded or truncate leaf base. Inflorescence supra-axillary or cauliflorous, flat and green-yellow flowers (Figure 19).

Pharmaceutical properties

This plant is used for treatment of wounds, ulcers, skin diseases, diabetes, fever, inflammation, fever, herpes and microbial infection.

Bioactive constituents

Berberine, alkaloid, noroxyhydrastine, dihydroberlambine, isoquinoline alkaloid were found as bioactive constituents (Chitra *et al*., 2011; Ramasubbu *et al*., 2012; Tungpradit *et al.*, 2010).

Figure 19 *Coscinium fenestratum* (Gaertn.) Colebr. (Tushar *et al*., 2008)

15.4 *Croton roxburghii* N.P. Balakr.

Family: *Euphorbiaceae*

Characteristics

This plant has smooth grey bark with height of 8 m. Leaf is simple, ovate or lanceolate, 5-10 cm wide, 9-30 cm long. Flowers are greenish yellow. Inflorescence is in terminal raceme or panicle; unisexual, monoecious or dioecious (Figure 20).

Pharmaceutical properties

This plant is used for treatment of dysmenorrhea, dysenteria, cancer and chronic hepatitis.

Bioactive constituents

Diterpenoid, furanocembranoid, croblongifolin and crotohalimaneic acid were found as bioactive constituents (Jansakul *et al*., 1997; Rukachaisirikul *et al*., 2002; Suwancharoen *et al*., 2012).

Figure 20 *Croton roxburghii* N.P. Balakr.

15.5 *Derris scandens* (Roxb.) Benth.

Family: *Leguminosae*

Characteristics

This plant has large evergreen climbing branched shrub. Leaves are compound, alternate simple pinnate with 7-13 opposite leaflets. Flowers are in short branches, bisexual and whitish or pinkish color. Fruits are narrowly oblong with a broad wing along one side. Inflorescence is a raceme of pea-like flowers, twice as long as the leaves (Figure 21).

Pharmaceutical properties

This plant is used for treatment of diarrhea, muscle ache, diuretic, arthritis and microbial infection.

Bioactive constituents

Coumarins, chandalone, beta-sitosterol, lupeol, glycosides, isoflavone, flavonoids, lonchocarpic acid, scandenone were found as bioactive constituents (Falshaw *et al*., 1969; Mahabusaraka *et al.,* 2004; Voravuthikunchai *et al*., 2004).

Figure 21 *Derris scandens* (Roxb.) Benth.

15.6 *Eclipta prostrata* (L.) L.

Family: *Asteraceae*

Characteristics

This plant is multibranched herb, 20–90 cm height with cylindrical and grayish root at the nodes. Leaves are 2.5-7.5 cm long, opposite, sessile, lanceolate, pilose, oblong to lanceolate and remotely serrate. Stems are reddish-purple with short, flat and up-turned hairs. Solitary flower heads are 6-8 mm in diameter and white floret (Figure 22).

Pharmaceutical properties

This plant is used for treatment of skin diseases, microbial infection, enteritis and atherosclerosis.

Bioactive constituents

Triterpene, oleanolic acid, polyacetylenes, flavonoid, sapronin, ecliptasaponin A, polypeptides, merulinic acid, steroids, eclalbatin, rusolic acid, nicotine, alpha-amyrin, daucoterol, β-amyrone were found as bioactive constituents (Jayathirtha and Mishra, 2004; Pithayanukul *et al*., 2004; Tewtrakul *et al*., 2011).

Figure 22 *Eclipta prostrata* (L.) L. (Johnsirani *et al*., 2013)

15.7 *Glycyrrhiza glabra* (L.)

Family: *Fabaceae*

Characteristics

This plant is perrineal or subshrub herb that attains 2 m height and sweet taste. Root color is grey-brown exterior and yellow interior. Leaves are alternate, pinnate, yellow green with a single leaflet pointing outwards at the end. Flowers appear in axillary spikes and purple color (Figure 23).

Pharmaceutical properties

This plant is used for treatment of inflammation, microbial infection, sore throat, fever, skin disease, arthritis, dyspepsia, herpes, bronchitis and rheumatic disease.

Bioactive constituents

Glycyrrhizin, prenylated biaurone, isoliquiritigenin, coumarin, licoisoflavone, alkaloids, hispaglabridins, glabridin, licochalcone A, saponins, isoflavone were found as bioactive constituents (Gupta *et al*., 2008; Maurya *et al*., 2009; Vispute and Khopade, 2011).

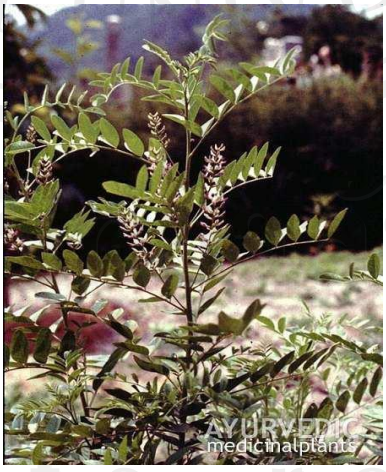

Figure 23 *Glycyrrhiza glabra* (L.) (Visput. and Khopade, 2011)

15.8 *Gynostemma pentaphyllum* (Thunb.) Makino

Family: *Cucurbitaceae*

Characteristics

This plant is a climbing vine. Stem and branches are slender, angular-sulcate and glabrous. Leaves are sedately, membranous and glabrous. Leaflets are ovate-oblong or lanceolate, small lateral leaflets. Fruit is indehiscent, black when mature and globose (Figure 24).

Pharmaceutical properties

This plant is used for treatment of cancer, ulceration, diabetes, inflammatory, tumor, hyperglycemic, asthma, chronic, fatigue and hepatitis.

Bioactive constituents

Gypenosides, flavonoids, phenolics, triterpenoids isoglabrolide, glabrin A glycyrrhizin, triterpene glycyrrhetol, coumarins, isoflavones, sterols, sapronin and glabrolide were found as bioactive constituents (Bai *et al.,* 2010; Wang *et al*., 2012; Xie *et al*., 2012; Zhao *et al*., 2012).

Figure 24 *Gynostemma pentaphyllum* (Thunb.) Makino (Mishra and Joshi, 2011)

15.9 *Houttuynia cordata* Thunb.

Family: *Saururaceae*

Characteristics

This plant is herbaceous perennial ground cover plant with 30-60 cm height. The stems are green or sometimes purplish red. The leaves are alternate, broad heart-shape leaves and purple underneath. Flowers are small, densely clustered on short spikes and greenish-yellow (Figure 25).

Pharmaceutical properties

This plant is used for treatment of microbial infection, inflammation, cancer, allergy, anaphylaxis, pneumonia, edema, lung abscess and dyspnea.

Bioactive constituents

Flavonoids, methyl nonyl ketone, camophyllene, bornyl acetate, α-pinene, β-pinene, a-terpineol, tannin, pectic-like substance, sabinene, glycones and phenolic were found as bioactive constituents (Kim *et al*., 2012; Lau *et al.,* 2008; Ni *et al*., 2007; Wei *et al*., 2011).

Figure 25 *Houttuynia cordata* Thunb.

15.10 *Leptochloa chinensis* (L.) Nees

Family: *Poaceae*

Characteristics

This plant is strongly tufted or perennial grass and 120 cm in height. Leaf sheaths are glabrous, smooth and linear. Stems are slender, hollow, erect or ascending from a branching base. It has an inflorescence forms loose pendulous panicle, narrowly ovate and flexuous branches. Its flowering culms are erect or ascending from a branching base (Figure 26).

Pharmaceutical properties

This plant is used for treatment of diarrhea, gastrointestinal irritation, tumor, cancer and inflammation.

Bioactive constituents

Phenolic and flavonoid were found as bioactive constituents (Rungprom and Prasantawona, 2009).

Figure 26 *Leptochloa chinensis* (L.) Nees (Akanksha, 2009)

15.11 *Momordica charantia* Linn.

Family: *Cucurbitaceae*

Characteristics

This plant is climbing perennial with elongated white or green fruit and has a bitter taste. Stems are angled and grooved. Alternate leaves are orbicular or reniform, lobes ovate-oblong, acute or sub-acute and apiculate**.** Flowers are monoecious. Seed is oblong, sub-bidentate at base and apex, sculptured on sides, cream or grey-color (Figure 27).

Pharmaceutical properties

This plant is used for treatment of diabetes, obesity, cancer, microbial infection, analgesic, inflammation, fertility, fever, leprosy, hypotensive and stomachache.

Bioactive constituents

Chorine, goyaglycosides, flavonoids, momordicilin, phenolic, goyasaponins, cucurbitanes, glycosides, galacturonic acid, triterpenes and momorcharins were found as bioactive constituents (Chiampanichayakul *et al.*, 2001; Gupta *et al.*, 2011; Lee *et al.*, 2009; Wu and Ng, 2008).

Figure 27 *Momordica charantia* Linn. (Gupta *et al.*, 2011)

15.12 *Phyllanthus amarus* Schum.&Thonn.

Family: *Euphorbiaceae*

Characteristics

This plant is erect herb with 30–40 cm height and bears ascending herbaceous branches. The bark is smooth and light green. Leaves are pale green, 6-8 mm wide, 3-4 mm long, elliptic to oblong. Flowers are pale green, which are often flushed with red. Fruits are tiny and smooth capsules containing seeds (Figure 28).

Pharmaceutical properties

This plant is used for treatment of diabetes, otitis, diarrhea, swelling, gastrointestinal disturbances, skin disease, cancer, hypertension, jaundice and nociceptive.

Bioactive constituents

Alkaloids, phyllnirurin, cardiac glycodises, flavonoids, amarulone, terpenes, tannin, phenols, steroids and saponins were found as bioactive constituents (Joseph and Raj, 2011; Nikam *et al*., 2011; Obianime and Uche, 2009).

Figure 28 *Phyllanthus amarus* Schum.&Thonn

15.13 *Pluchea indica* (Linn.) Less.

Family: *Asteraceae*

Characteristics

This plant has rich branch shrubs or half-shrubs, ribbed smooth and fluffy with 2 m height. Short-stemmed leaves are alternate pale green, round egg shape breech and the round ends tapering. Flowers are pink-purple color and leaf-shaped flowers in axillary or sit-handled bulb. Fruit is brown with white corners (Figure 29).

Pharmaceutical properties

This plant is used for treatment of dysentery, ulcers, inflammation, fever, bad breath, body odor, abdominal pain and rheumatism.

Bioactive constituents

Phenolic, glycosides, flavonoids, triterpenes and steroids were found as bioactive constituents (Andarwulan *et al.*, 2010; Burger *et al.*, 2000; Chang *et al.*, 2012; Noridayu *et al.*, 2011)

Figure 29 *Pluchea indica* (Linn.) Less.

15.14 *Pseuderatherum platiferum* (Wall.) Radlk. ex. Lindau.

Family: *Acanthaceae*

Characteristics

This plant has small shrub stems with 1-2 m in height. Bark is smooth and green. Leave is green, 3-5 cm wide and 10-20 cm long, semi-oval, lanceolate, base unequal sided, apex obtuse to acute. Flowers are purplish white (Figure 30).

Pharmaceutical properties

This plant is used for treatment of diarrhea, sore throat, inflammation, rheumatoid and heart disease.

Bioactive constituents

Flavonoids, stigmasterol, 1-triacontanol, glycerol-1-hexandecanoate and salicylic acid were found as bioactive constituents (Dieu *et al*., 2005; Sriruvoramas *et al*., 2008).

Figure 30 *Pseuderatherum platiferum* (Wall.) Radlk. ex. Lindau. (Srisuvoramas *et al*., 2008)

15.15 *Rhinacanthus nasutus* (Linn.) Kurz

Family: *Acanthaceae*

Characteristics

This plant is erect shrubs and 1-1.5 m in height. Stem are terete and tomentose. Leaves are oblong, 8-12 cm long and 4-8 cm wide, elliptic and acute at both ends. Flower is linear-lanceolate, corolla white, slender and hispid. Upper lip is entire, oblong and acuminate, and lower lip is broad (Figure 31).

Pharmaceutical properties

This plant is used for treatment of virus infection, fungal infection, cancer, skin diseases, peptic ulcers, inflammation, hepatitis, ringworm and eczema.

Bioactive constituents

Naphthoquinones, sterols, quinone, anthraquinone, flavonoids, benzenoids, coumarin, triterpenes, glycosides and terpenoids were found as bioactive constituents (Rao *et al*., 2010; Siripong *et al*., 2006; Suman *et al*., 2011; Tewtrakul *et al*., 2009).

Figure 31 *Rhinacanthus nasutus* (Linn.) Kurz

15.16 *Schefflera leucantha* R.Vig.

Family: *Araliaceae*

Characteristics

This plant has shrub highly branched with 1-2 m in height. The finger-like leaf composed of 5-6 compound leaves, oblanceolate-subulate and glabrous. Flowers are white, small and inflorescence. Fruits are yellow and dense aggregate (Figure 32).

Pharmaceutical properties

This plant is used for treatment of chronic asthma, microbial infection, hypoglycemic, inflammation, nosocomial infection, allergies, respiratory tract, respiratory infection, peptic, analgesics, bronchitis and cancer.

Bioactive constituents

Saponin, steroids, terpenes and flavonoids were found as bioactive constituents (Matsui *et al.,* 2010; Potduang *et al*., 2007; Sittiwet *et al.,* 2009).

Figure 32 *Schefflera leucantha* R.Vig.

15.17 *Senna alata* (Linn.) Roxb.

Family: *Febaceae*

Characteristics

This plant has shrub with 3–4 m in height. Leaves are paripinnate, 30 cm wide, 60 cm long. Leaflet is oblong or elliptic oblong and glabrous. Yellow flowers are densely axillary racemes. Fruit shaped like a straight pod, thick, flattened, wing and glabrous (Figure 33).

Pharmaceutical properties

This plant is used for treatment of microbial infection, parasitic skin disease, haemorrhoids, constipation, syphilis, diabetics, fungus infection, convulsion and gonorrhea.

Bioactive constituents

Anthraquinones, tannins, flavonoids, triterpenoids, sterols, fatty acid, saponins, glycoside, polyphenol and alkaloids were found as bioactive constituents (Mohideen *et al.*, 2005; Panichayupakaranant and Intaraksa, 2003; Sule *et al.*, 2010).

Figure 33 *Senna alata* (Linn.) Roxb. (Brown, 2013)

15.18 *Sphenodesme* sp.

Family: *Verbenaceae*

Characteristics

This plant has height of 4.5 m shrub plant. Stems are squarish or terete, lenticelled and glabrous. Branches are pilose. Leaves are simple, opposite, oblong ovate, green above and shining pale below. Fruits are 3-winged (Figure 34) (Tuuk, 1866; Wiart, 2006).

Figure 34 *Sphenodesme* sp.

15.19 *Stemona tuberosa* Lour.

Family: *Stemonaceae*

Characteristics

This plant has perennial herb and 4-10 m in height. Stems are often branched and woody base. Leaves are arranged opposite or whorled, alternate, ovate or broadly ovate. Inflorescence is axillary and raceme-like (Figure 35).

Pharmaceutical properties

This plant is used for treatment of pulmonary tuberculosis, microbial infections, bronchitis, pertussis, fungus infection, tumor, carcinoma and cough.

Bioactive constituents

Tocopherols, stilbenoids and alkaloids were found as bioactive constituents (Brem *et al.*, 2002; Jiang *et al*., 2006; Kakuta *et al.*, 2003; Li *et al.*, 2007).

Figure 35 *Stemona tuberosa* Lour.

15.20 *Stephania venosa* (Blume) Spreng.

Family: *Menispermaceae*

Characteristics

This plant has herbaceous perennial vines with 4 m in height. Stem or woody are glabrous or puberulous. Root is tuberous. Leaves are arranged spirally on stem, simple and entire, peltate, petiole usually geniculate at base, and inflorescence axillary or cauliflorous. Flowers are unisexual (Figure 36).

Pharmaceutical properties

This plant is used for treatment of inflammation, sores, microbial infection, cancer, stomachache, disease, wounds and leprosy.

Bioactive constituents

Cardiac glycosides, vitexin, apigenin, lignin, isovitexin, terpenoids, tannins, saponin, alkaloid, jatrophine, phenolic, steroids and flavonoids were found as bioactive constituents (Dhale and Birari, 2010; Gomuttapong *et al*., 2012; Leewanich *et al*., 2011; Seth and Sarin, 2010).

Figure 36 *Stephania venosa* (Blume) Spreng.

15.21 *Thunbergia laurifolia* Lindl.

Family: *Acanthaceae*

Characteristics

This plant is vigorous perennial evergreen climbing vine, hairless and reaching a length of 12 m. Heart-shaped with serrated leaf margin, oval and narrow that grow in opposite pairs. Flowers are large trumpet-shaped, white outside and yellowish inside. Seed capsules are brown in cone shaped pods (Figure 37).

Pharmaceutical properties

This plant is used for treatment of inflammation, pyretic, microbial infection, mutagenic, cancer and detoxification of poison.

Bioactive constituents

Glucosides, glucosides, phenolic, iridoid flavonoid C-glycosides and benzyl alcohol were found as bioactive constituents (Chan and Lim, 2006; Palipoch *et al*., 2011; Thongsaard *et al*., 2005).

Figure 37 *Thunbergia laurifolia* Lindl.

15.22 *Tinospora crispa* (Linn.) Miers ex Hook.f. & Thomson

Family: *Menispermaceae*

Characteristics

This plant is a deciduous vines herb. Old stems are fleshy, with very prominent blunt tubercles. Younger stems are slightly fleshy, brownish and glabrous. Leaf is large lenticels, broadly ovate to orbicular, slightly fleshy and rounded lobes (Figure 38).

Pharmaceutical properties

This plant is used for treatment of wounds, skin infection, hypertension, carcinogenic, inflammation, dysentery, leprosy, allergic, diarrhea, heart diseases, rheumatoid arthritis, cancer and diabetes.

Bioactive constituents

Polyphenollic, alkaloid, glucoside, picroretoside, palmatine, steroids, flavonoids and triterpenes were found as bioactive constituents (Kadir *et al*., 2011; Patel *et al*., 2013; Sinha *et al*., 2004; Zulkhairi *et al*., 2008).

Figure 38 *Tinospora crispa* (Linn.) Miers ex Hook.f. & Thomson (Patel *et al*., 2013)

15.23 *Zingiber montanum* (Koenig) Linkex Dietr.

Family: *Zingiberaceae*

Characteristics

This plant is herbaceous plant with underground rhizome and 0.7-1.5 m in height. It has palmately paralled venation simple leaves, 3.5-5.5 cm long and 18-35 cm wide, lanceolate, apiculate and alternate distichous. Petals are creamy white and fruit is purple color (Figure 39).

Pharmaceutical properties

This plant is used for treatment of inflammation, rheumatism, muscular pain, wounds, asthma, microbial infection, cancer, joint pain, rheumatism, insecticide and fungal infection disease.

Bioactive constituents

Flavonoids, saponins, sesquiterpene, triterpene, curcuminoid, sabinene, triquinacene 1,4-bis, phenylnutenoid, phenolic and diterpene were found as bioactive constituents (Chairul *et al.*, 2009; Chaiwongsa *et al.*, 2012; Iswantini *et al.*, 2011; Lu *et al.*, 2008).

Figure 39 *Zingiber montanum* (Koenig) Linkex Dietr.