

Table of Content

1. INTRODUCTION	1
1.1. INFLUENZA A VIRUSES	1
1.1.1. Classification, genome, and structure	1
1.1.2. Influenza viruses replication cycle	1
1.1.3. Pathogenicity of avian influenza H5N1 in humans	2
1.1.4. Epidemiology of influenza viruses	6
1.2. ANTIVIRAL DRUGS FOR INFLUENZA VIRUSES.....	8
1.2.1. License antiviral drugs	9
1.2.2. Development of antiviral compounds.....	11
1.3. TARGETING THE HOST TO INHIBIT INFLUENZA VIRUSES INFECTION	13
1.3.1. Influenza virus entry	13
1.3.2. Influenza viral replication	14
1.3.3. Influenza viruses induced intracellular signalling pathways	15
1.3.3.1. PI3K/AKT pathway	15
1.3.4. Immunomodulatory approaches	17
1.3.5. Combination treatment.....	19
1.4. THE FLAVONOIDS	19
1.4.1. Origin and chemical structure	19
1.4.2. Classification.....	20
1.4.3. Biological activities	21
1.4.4. Biochanin A	23
1.4.5. Baicalein	23
1.5. PURPOSE OF STUDY	24
2. MATERIALS AND METHODS.....	25
2.1. MATERIALS	25
2.1.1. Chemicals.....	25
2.1.2. Buffers, solutions and culture media	26
2.1.3. Antibodies and Chromogens	31
2.1.4. Commercial reagents and Assay kit	32
2.1.5. Cell lines and primary cell culture	32
2.1.6. Influenza strains	33
2.1.7. Flavonoids.....	33
2.1.8. Antiviral drugs and anti-inflammatory agents	36
2.1.9. Software	37
2.1.10. BSL3 laboratory	37
2.2. METHODS	37
2.2.1. Cell culture preparation	37
2.2.2. Primary cell culture	37
2.2.3. Virus stock preparation	39
2.2.4. Virus infection	39
2.2.5. Standard end point titration assay	39
2.2.6. Cell viability assay	40
2.2.7. Antigen reduction assay	42
2.2.8. Immunohistochemistry staining	42
2.2.9. Cytotoxicity dose 50 (CC₅₀), inhibitory dose 50 (IC₅₀) and selective index (SI)	42
2.2.10. Virus yield reduction assay	43
2.2.11. Enzyme-Linked Immunosorbent Assay (ELISA)	43

2.2.12. <i>Cell free caspase-3 enzyme inhibition assay</i>	45
2.2.13. <i>Immunofluorescence staining</i>	46
2.2.14. <i>Protein extraction and western blot analysis</i>	47
2.2.15. <i>Neuraminidase inhibition (NAI) assay</i>	50
2.2.16. <i>Reactive oxygen species (ROS) production assay</i>	50
2.2.17. <i>Statistical analysis</i>	51
3. RESULTS	52
3.1. SELECTION OF POTENT FLAVONOIDS WITH H5N1 INFLUENZA A VIRUSES ANTIVIRAL ACTIVITIES	52
3.1.1. <i>Effects of the flavonoids to A549 cell viability</i>	52
3.1.2. <i>Screening of the flavonoids for H5N1 influenza A viruses antiviral activities</i>	52
3.1.3. <i>Cytotoxicity50 (CC_{50}), inhibitory50 (IC_{50}) and selective indices of 22 flavonoids against H5N1 influenza A viruses</i>	53
3.1.4. <i>Dynamics of biochanin A and baicalein on cellular viability</i>	56
3.2. IDENTIFICATION OF ANTIVIRAL AND ANTI-INFLAMMATORY OF BIOCHANIN A AND BAICALEIN	56
3.2.1. <i>Effects of biochanin A and baicalein to H5N1 influenza viral replication in A549 cells</i>	56
3.2.2. <i>Effects of biochanin A and baicalein on H5N1-induced pro-inflammatory cytokine expression in A549 cell</i>	58
3.3. DETERMINATION OF MOLECULAR MECHANISMS OF BIOCHANIN A AND BAICALEIN AGAINST H5N1 INFLUENZA VIRUS INFECTION	61
3.3.1. <i>Effect of biochanin A and baicalein on H5N1-induced caspase-3 activation</i>	61
3.3.2. <i>Effects of biochanin A and baicalein on viral ribonucleoprotein (RNP) complex nuclear transport</i>	62
3.3.3. <i>Effects of biochanin A and baicalein on intracellular signalling proteins</i>	64
3.3.4. <i>Effects of biochanin A and baicalein on H5N1 influenza virus neuraminidase (NA) activity</i>	68
3.3.5. <i>Effect of biochanin A and baicalein to reactive oxygen species (ROS) production</i>	70
3.4. EFFECTS OF BIOCHANIN A AND BAICALEIN IN COMBINATION TREATMENT	72
3.4.1. <i>Combination of biochanin A or baicalein and zanamivir</i>	72
3.4.2. <i>Combination of biochanin A or baicalein and N-acetyl-L-cysteine</i>	73
3.5. EFFECTS OF BIOCHANIN A AND BAICALEIN IN MONOCYTE-DERIVED MACROPHAGES (MDMs)	76
3.5.1. <i>Effects of biochanin A and baicalein on H5N1 viral replication in MDMs</i>	76
3.5.2. <i>Effects of biochanin A and baicalein on cytokine production in MDMs</i>	76
3.5.3. <i>Combination treatment of biochanin A and baicalein and NAC in MDMs</i>	77
3.6. EFFECT OF BIOCHANIN A AND BAICALEIN ON H5N1 INFLUENZA A/VIETNAM/1203/04 (A/VN/04)	80
4. DISCUSSION	83
5. CONCLUSION	91
REFERENCES	101
6. SUPPLEMENTARY DATA	116
6.1. SUPPLEMENTARY 1: FLAVONOIDS WITH NA INHIBITORY POTENTIALS	116
6.2. SUPPLEMENTARY DATA 2: CELLULAR VIABILITY ASSAYS AND THEIR VALIDITIES	118
ABBREVIATION LIST	120
FIGURE LEGENDS	122
TABLES	124
PUBLICATIONS	125
CURRICULUM VITAE	126
DECLARATION	128