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Immunomodulator From Andrographis Paniculata In Silico Approach Through Docking Análysis And Admet Predictions

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Abstract

One of the medicinal plants which has activity as an immunomodulator is bitter Andrographis paniculata. The immunomodulatory activities of Andrographis paniculata extract and its components (Andrographolide) is to decreased secretion of Interleukin-2. This study aimed to discover bioactivity of Andrographolide compound from Andrographis paniculata for immunomodulatorbased on reverse docking studies. Structures of chemical constituents of Andrographis paniculata (Andrographolide) was collected from published literature. The water molecule and ligands were removed by using PyMOL v1.7.4.5 Software (Schrödinger). Molecular docking experiments were performed using the PyRx 0.8 software. Prediction and significant descriptors of Physicochemical Properties, Lipophilicity, Pharmacokinetics and Druglikeness properties of the compounds were predicted using Swissadme. Andrographolide has greater potential as an immunomodulator compared to Muramyl dipeptide based on its binding affinity and intermolecular interactions. The binding affinity of Andrographolide with interleukin-2 protein is -6.9, while binding affinity interleukin-2 with the control compoundMuramyl dipeptide is -5.6. AMES Test showed that Andrographolide is not potential mutagens and not carcinogens. Druglikeness prediction showed that Andrographolide fulfil the rules of Lipinski, Ghose, Veber, Egan and Muegge with 0.55 Bioavailability Score.

Keywords: Immumodulator, Andrographis paniculata,Andrographolide, Interleukin-2

Introduction

Benefits ofandrographis paniculata plants against the body's immune system.The immune system is a collection of mechanisms in a living thing organism as a form of protection infection by identifying and killing pathogenic substances. Cells involved in the immune system in the body are T-cells produced by the thymus and B cells produced in the spinal cord. The

development and activity of T cells can be stimulated by the addition of an immunomodulator. Andrographis paniculata contains deoxyandrographolide, andrographolide, 14-deoxy-11, neoandrographolide, 12- didehydroandrographolide, homoandrographolide, diterpenoid and flavonoid. Andrographis paniculata has an active compound called andrographolide, where these compounds acts as immunomodulators, especially immunostimulants which can increase the work of the immune system. The content of Andrographolide can improve the function of the body's defense system such as white blood cells to attack bacteria and other antigens (immunomodulators), flavonoids as anti-inflammatory, and tannins as antidiarrheals. Andrographis paniculata plant can also be an immunosupressor which can decrease the body's immune response when the immune system increases beyond normal body conditions. But until now, unwanted side effects are rarely encountered during the utilisation of andrographis paniculata (Riska, 2020).

Andrographolide also exhibits immunomodulatory effects by effectively enhancing cytotoxic T cells, natural killer (NK) cells, phagocytosis, and antibody-dependent cell-mediated cytotoxicity (ADCC). All these properties of andrographolide form the foundation for the use of this miraculous compound to restrain virus replication and virus-induced pathogenesis (Swati Gupta, 2017).

One of the medicinal plants which has activity as an immunomodulator is bitter Andrographis paniculata. The immunomodulatory activities of andrographis paniculata extract and its components (Andrographolide)is to enhanced proliferation and interleukin-2 (IL-2) induction human peripheral blood lymphocytes (HPBLs) (R. Ajaya K, 2004)

Andrographolide is a diterpenic labdane that possesses anti-inflammatory and immunomodulatory effects. Several studies propose that andrographolide can reduce the immune response through inhibition of the nuclear factor kappa B (NF-κB) and mitogen-activated protein kinases (MAPK) such as extracellular signal regulated kinase 1/2 (ERK1/2) pathways. Moreover, andrographolide reduces IFN-γ and IL-2 production induced by concanavalin A in murine T-cell (Maria D. Carretta, 2009)

Andrograholide was reported to have immunoregulatory activities. In tumor-bearing mice it enhanced natural killer cell activity, increased secretion of IL-2 and IFN-γ by T cells and thereby inhibited the tumor growth. In autoimmune encephalomyelitis mice it interfered with maturation of dendritic cells, induced antigen-specific tolerance and thus prevented detrimental autoimmune responses. It suggests that andrographolide can have different effects in different immune disease models, playing a role as a modulator of altered immune responses rather than a sole immunostimulatory or immunosuppressive agent. Considering that macrophages are widely distributed immune cells that play an indispensable role in homeostasis and defense, we investigated the immunomodulatory effect of andrographolide on the alteration of macrophage phenotype and function (Wei Wang, 2010).

Interleukin-2 produced by T-cells in response to antigenic or mitogenic stimulation, this protein is required for T-cell proliferation and other activities crucial to regulation of the immune response. Can stimulate B-cells, monocytes, lymphokine-activated killer cells, natural

killer cells, and glioma cells (Hideki Kitaura, 2018).Muramyl dipeptide (MDP), the minimal essential structural unit responsible for the immunological activity of peptidoglycans, is another inflammation-inducing molecule that is ubiquitously expressed by bacteria. Several studies have shown that inflammation-related biological activities were synergistically induced by interactions between LPS and MDP. MDP synergistically enhances production of proinflammatory cytokines that are induced by LPS exposure (Maria M.J.P Willems, 2007).

Materials and Method

Ligands Preparation

Structures of the chemical compound of Andrographolide was collected from published literature. Chemical 3D structure and SMILES of ligand (Andrographolide) taken from PubChem compound database (https://pubchem.ncbi.nlm.nih.gov/) with number ID: CID 5318517 and Canonical Smile: CC12CCC(C(C1CCC(=C)C2CC=C3C(COC3=O)O)(C)CO)O. The two-dimensional (2D) and the three-dimensional (3D) chemical structures of the ligands were sketched using Avogadro and Discovery Studio and were saved in PDB format

Target Selection

The protein potential target candidates for docking was prepared using 3 databanks, i.e: Pharmmapper (http://lilab.ecust.edu.cn), SuperPred (http://prediction.charite.de), and Swiss Target Prediction (www.swisstargetprediction.ch) and validate using Uniport (https://www.uniprot.org). The protein that was collected and validated with PDB (Protein Data Bank https:///www.rcsb.org/pdb) than proteins were prepared using clean protein to remove the water molecules from the structure. The water molecule and ligands were removed by using PyMOL v1.7.4.5 Software (Schrödinger). In this study, the target protein used Interleukin-2 code of PDB, because Interleukin-2 is a compound in the form of an protein that plays an essential role as an immunostimulatory factor that supports the expansion of activated effector T cells.

Molecular Docking

Molecular docking experiments were performed using the PyRx 0.8 software. The reverse docking process was carried out using the Vina Wizard feature integrated into PyRx 0.8 software which reacts to the natural compound Andrographolide, the target protein Interleukin-2 and the control compound (activator compound Muramyl dipeptide). Activator compounds will be a positive control in the docking process. The activator compound of Interleukin-2is Muramyl dipeptide. Muramyl dipeptide (MDP) is the smallest peptidoglycan fragment capable of triggering the innate immune system through interaction with the intracellular NOD2 receptor (Sulfahri, 2019).

Visualization of Molecule and Small Molecule Interaction

The interactions between ligands (Andrographolide) target protein (Interleukin-2), and known inhibitors of target protein (Muramyl dipeptide) visualized and analyzed using PyMol v1.7.4.5 Software (Schrödinger)

Compound's Properties and ADMET Predictions

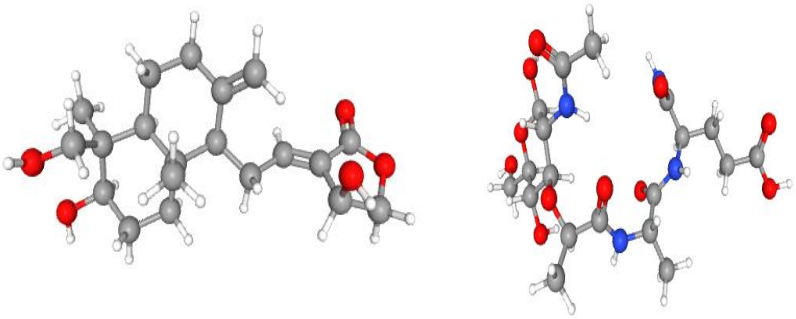
Swissadme (http://www.swissadme.ch) and admetSAR (lmmd.ecust.edu.cn:8000) is used to predict the prediction and significant descriptors of Physicochemical Properties, Lipophilicity, Pharmacokinetics and Druglikeness properties of the compounds.

Results and Disscussion

The main compounds found in Andrographis paniculatais Andrographolide. Andrographolide compounds are known to interact with one type of protein in macrophages.Based on PharmMapper target prediction result which has been found that Andrographolide has a connection with an protein of cell macrophages. One type of protein is Interleukin-2. Interleukin-2 plays an important role modulator of macrophage. Interleukin-2 is protein secretion of T cells. The activator compounds of Interleukin-2 is Muramyl dipeptide .

Structure of natural compounds with control compounds and target proteins, visualized in 3 dimensions (3D) using PyMol. Through reverse docking technique can be known the potential of an Andrographolide has the potential as immunomodulator. Interaction of Andrographolide with Interleukin-2 compared to Muramyl dipeptide as the control compound. Based on reverse docking results, the binding affinity of interleukin-2 protein to Muramyl dipeptideshowed lower binding affinity than interleukin-2 protein to andrographolide.

The number of binding affinities illustrate the potential of a compound or a ligand to interact with its protein (protein target). If the ligand has lower binding affinity, it will be stronger to inherit the protein target.Hence, the lower binding affinity leads the lower energy needed of ligand to interact with the protein target(Antonella Di Sotto, 2020). The interleukin-2 protein plays a role in the regulatory process of immunomodulatory has an interaction with natural compounds from the Andrographis paniculata plant that has been visualized in 3D in PyMol software. The binding affinity of Andrographolide with interleukin-2 protein is -6.9, while binding affinity interleukin-2 with the control compound Muramyl dipeptide is -5.6. Based on the result, comparing the strengthen of Andrographolide with Muramyl dipeptide to interleukin-2 has shown that Andrographolide has an ability in attaching the protein of macrophage.



(A) (B)

Figure 1. (A) Chemical 3D Structure of Andrographolide and (B) Muramyl dipeptidewere showed by software PyMol

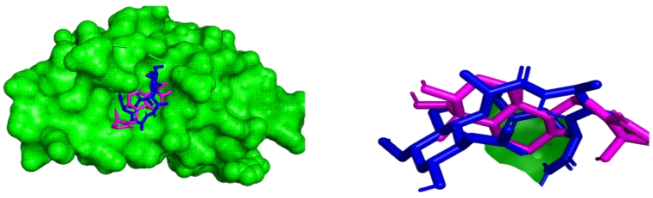


Figure 2. Binding Site of Andrographolide(purple), Muramyl dipeptide(blue) with Interleukin-2 (green)

Table 1. The result of Reverse Docking Interleukin-2 with ligand and control activator

Ligand	Binding Affinity
Interleukin-2 and Andrographolide	-6.9
Interleukin-2 and Muramyl dipeptide	-5.6

Most of the drugs have aimed for treating some chronic diseases. Thus, the concentration of a drug must be consistent.[7] The side effect of the Andrographolidecompound for the body has observed by ADMET predictions which were evaluated and linked to cell permeation, metabolism process and bioavailability. As revealed by the result of this study (AMES Test), the result shows that Andrographolideis not potential mutagens and not carcinogens. The Ligands is considered to have the potential to enter the cell membrane and be absorbed by the body if they meet Lipinski's rules. The search results show that AndrographolideGI absorption high, fulfils the rules of Lipinski, Ghose, Veber, Egan and Muegge with the Bioavailability Score 0.55.The search result Muramyl dipeptide GI absorption low, doesn't meet the rules of Lipinski, Ghose, Veber, Egan and Muegge with the Bioavailability Score 0.11.

Excretion		
Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9382
	Inhibitor	0.5000
AMES Toxicity	Non AMES toxic	0.8714
Carcinogens	Non-carcinogens	0.9618
Fish Toxicity	High FHMT	0.9873
Tetrahymena Pyriformis Toxicity	High TPT	0.9827
Honey Bee Toxicity	High HBT	0.8182
Biodegradation	**Not ready biodegradable**	0.9535
Acute Oral Toxicity	III	0.5328
Carcinogenicity (Three-class)	Non-required	0.5856
ADMET Predicted Profile --- Regression		

Figure 3. ADMET predictions Andrographolide

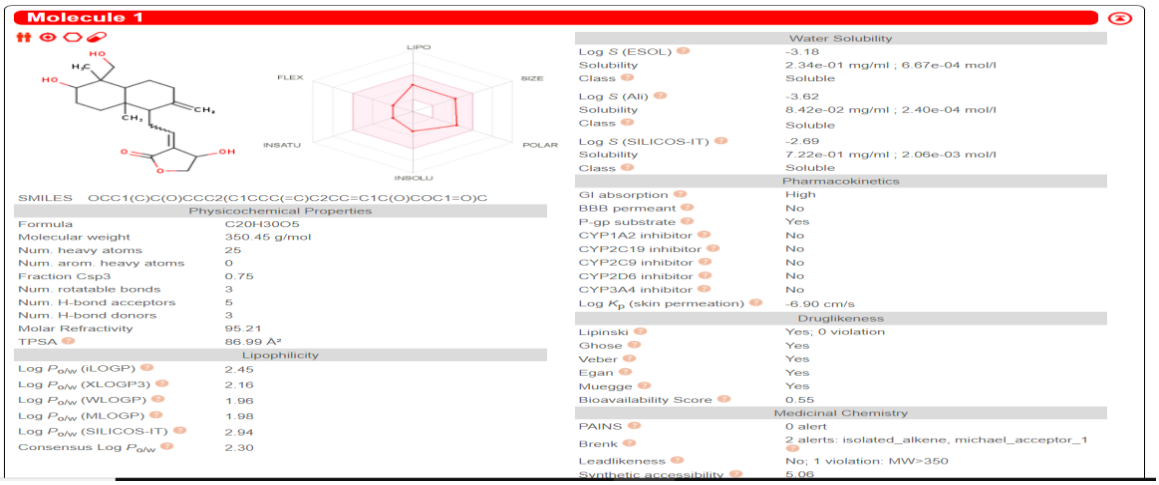


Figure 4. Physicochemical Properties, Lipophilicity, Pharmacokinetics and Druglikeness properties of Andrographolide

Andrographis paniculata (Burm.f.) Wall. ex Nees., (Family-Acanthaceae) (English name-King of Bitters, Tamil name-Nilavempu) is an annual herbaceous plant and is extensively cultivated in Southern Asia, China and some parts of Europe. In traditional medicine, A. paniculata is widely used to get rid of body heat, dispel toxins from the body; prevent common cold, upper respiratory tract infections including sinusitis and fever and as an antidote against poisons of snakes and insects (Samy RP, 2008). The plant has been reported to exhibit various mode of biological activities in vivo as well as in vitro viz., antibacterial (Mishara US, 2009; Parvataneni, 2010; Roy S, 2010, Abubakar S, 2011), antiviral, anti-inflammatory, anti HIV (Human immunodeficiency virus), immunomodulating/ immunostimulatoryand anticancer (Wen Wc, 2010; Chao WW, 2010). The plant showed potential therapeutic action in curing liver disorders, common cough and colds in human (Geethangili M, 2008). The characteristic secondary metabolites encountered in this plant have considerably enhanced its importance in the arena of medicinal plants (Joseph Joselin and Solomon Jeeva, 2014).

The published clinical studies on A. paniculata reported prophylactic and therapeutic benefits for upper respiratory tract infections, increase in immune cell counts in HIV infected patients, and anti-cancer effects. Also, several animal and cell-based studies are available to indicate broad spectrum anti-viral activity of the plant against viruses such as influenza A virus, dengue virus, chikungunya virus, human immunodeficiency virus, hepatitis B virus, hepatitis C virus, herpes simplex virus 1, EpsteineBarr virus, and human papillomavirus. The effects on the seasonal cold and flu virus and other viruses are suggestive that A. paniculata may be a modifiable factor in impacting immune function(Muhammad Torequl Islami, 2018).

Immune system protects the host from pathogenic organisms (virus, bacteria etc.) and helps in cell cycle homeostasis. Humans possess innate and adaptive immune responses that work synergistically and function in a highly orchestrated manner. The innate arm involves NK cells, phagocytes as a quick non-specific defense while the adaptive arm involves T and B lymphocytes. Another important component of the immune system is the cytokines that regulate the immune cells and has many other functions such as anti-viral activity. The immune responses are compromised due to stress, age, and lifestyle choices that render the

host vulnerable to infections. Hence, herbs/phytoactives that are biological response modifiers known to modulate immune function aids in protecting the host from infections (M. Rajanna A, 2021).

Two other main effects of Andrographolide are the inhibition of cell replication and migration. When Andrographis paniculata extract was further fractionated into aqueous, methane and ether fractions, three were found to inhibit the proliferation of cancer cells. The only non-active fraction was the aqueous one. Simultaneously, these fractions elevated proliferation of peripheral blood lymphocytes and induced IL-2 formation (Vaclav Vetvicka, 2021).

Standardized A. paniculata extract (SAPE) increased T cells, T helper cells and significantly increased IFN-g, IL-4, and decreased IL-2 at day 30. A subgroup analysis of participants with absolute lymphocyte counts of 1000e3000 cells/mm3 indicated that there is a significant increase in the T-cells, T-helper cells at day 7 and 30 and significant increase in IFN-g, IL-4 and decrease in IL-2 at day 30. There was no treatment related adverse effects following SAPE intake for 30 days (M. Rajanna A, 2021).

Interleukin-2 (IL-2) was first described as an immunostimulatory factor that supports the expansion of activated effector T-cells (Jonathan G Poll, 2020). Interleukin-2 (IL-2) exerts crucial functions during immune homeostasis via its effects on regulatory T (Treg) cells, and the optimizing and fine-tuning of effector lymphocyte responses. Thus, somewhat paradoxically, low doses of recombinant IL-2 have been used for Treg cell-based immunosuppressive strategies against immune pathologies, while high-dose IL-2 has shown some success in stimulating anti-tumor immune responses (Natalia Arenas-Ramirez, 2015)

Conclusion

This study proved that Andrographolidehas potential as an immunomodulator based on its binding affinity with -6.9 and intermolecular interactions. Andrographis paniculatacontains Andrographolide which is potential immunomodulator according to Lipinski, Ghose, Veber, Egan dan Muegge rule and 0.55 Bioavailability Score.

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Conflict of Interest.

The authors declare that there is no conflict of interest.

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