Molecular docking studies of lupeol and beta-sitosterol on Immunomodulatory receptors: Research

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Abstract- The objective of present work was to study the immunomodulatory potential of lupeol and beta-sitosterol by in silico approach. *In silico* models involved to study binding affinity of lupeol and beta-sitosterol molecules on immune modified proteins such as Toll like receptor and human tumor necrosis factor-alpha (TNF-α). Docking results showed that the lupeol and beta-sitosterol bioactive molecules were showed immune modulatory activity due to high binding affinity and H bonding interaction with active sites of Toll like receptors and TNF-α respectively as compared to standard immunomodulatory molecule levamisole. Based on docking lupeol and beta-sitosterol were concluded as immunomodulatory potential candidate.

***Index Terms*- immunomodulator.lupeol, beta-sitosterol, Toll like receptors, TNF-α.**

INTRODUCTION

Immunomodulation-

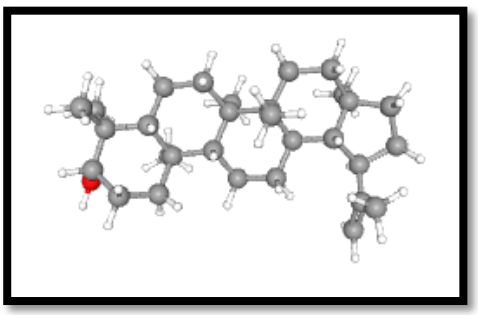
Immunomodulation is a broad word that refers to any changes in the immune response, including induction, expression, amplification, and inhibition of any part or phase of the immune system. Infections, organ transplantation, cancer, rheumatoid arthritis, systemic lupus erythematous, Down syndrome, Crohn's disease, and autoimmune illnesses, as well as the acquired immune deficiency syndrome, all require immunomodulating medications (AIDS). Furthermore, physiologically active chemicals derived from natural sources have long piqued researchers' curiosity when it comes to fighting infectious diseases or improving immune function. The identification and isolation of more selective immunomodulatory compounds from plant sources has a lot of promise in terms of reducing the high cost of synthetic molecules and their negative side effects.

Lupeol-

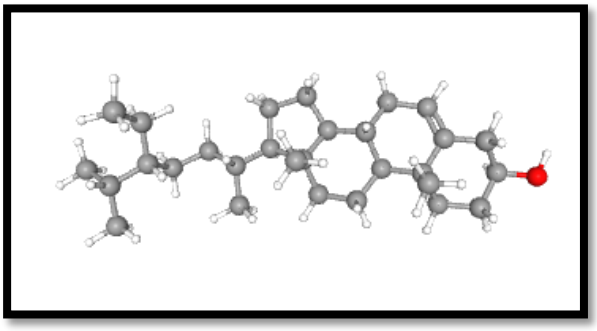
Lupeol is a phytosterol that is pharmacologically effective in treating a variety of illnesses, including cancer and microbial infections. Lupeol causes an increase in CD4+ and CD8+ T cell percentage. This CD8+ and CD4+ T cells play a critical role in the efficient control of infection, as these cells produce proinflammatory cytokines like IFN-γ and TNF-alpha, which trigger macrophages to release oxidative radicals like NO to destroy intracellular parasites. Additionally, it promotes monocyte recruitment by increasing the expression of the NF-κB gene, which is responsible for the generation of chemotactic factors, adhesion molecules, and pro-inflammatory cytokines. Lupeol alters the expression or function of a number of molecules, including the cytokines IL-2, IL-4, and IL-5. Numerous medicinal plants, including Bauhinia variegata, Euphorbia resinifera, and Sterculia villosa, contain lupeol, an efficient immunomodulatory triterpenoid.

Beta-sitosterol-

It has been demonstrated that beta-sitosterol has anti-inflammatory, anti-neoplasic, anti-pyretic, and immune-modulating properties. Beta-sitosterol appears to target particular T-helper (Th) lymphocytes, improving T-lymphocyte and natural killer (NK) cell activity while boosting Th1 activity. When beta-sitosterol activates dendritic cells, they may cause lymphocyte proliferation or stop lymphocytes from dying, and they may also cause the release of IFN-α. It has been shown that beta-sitosterol affects macrophages. The macrophages release a number of cytokines as a result, including IL-10, IL-6, IFN-γ, and MCP-1. It affects the anti-inflammatory effect on macrophages by activating protein tyrosine phosphatase (SHP-1). Additionally, it promoted the production of Th1-dominant cytokines by CD4+ T cells. Depending on the cellular response, Th1-type cytokines can help the host defend against viral and bacterial infections and avoid infection.



A



B

Fig.No.1. 3D structures of A. Lupeol, B. Beta-sitosterol

Toll like receptors and TNF-α receptors-

The best characterised innate receptors are toll-like receptors (TLRs), which are quickly activated and comprise functional modules that are essential for the host's defence against microbial invasion. They are single-pass membrane-spanning receptors usually expressed on sentinel cells such as macrophages and dendritic cells, that recognize structurally conserved molecules derived from microbes. TNF receptors are primarily involved in apoptosis and inflammation, but they can also take part in other signal transduction pathways, such as proliferation, survival, and differentiation. TNF receptors are expressed in a wide variety of tissues in mammals, especially in leukocytes.

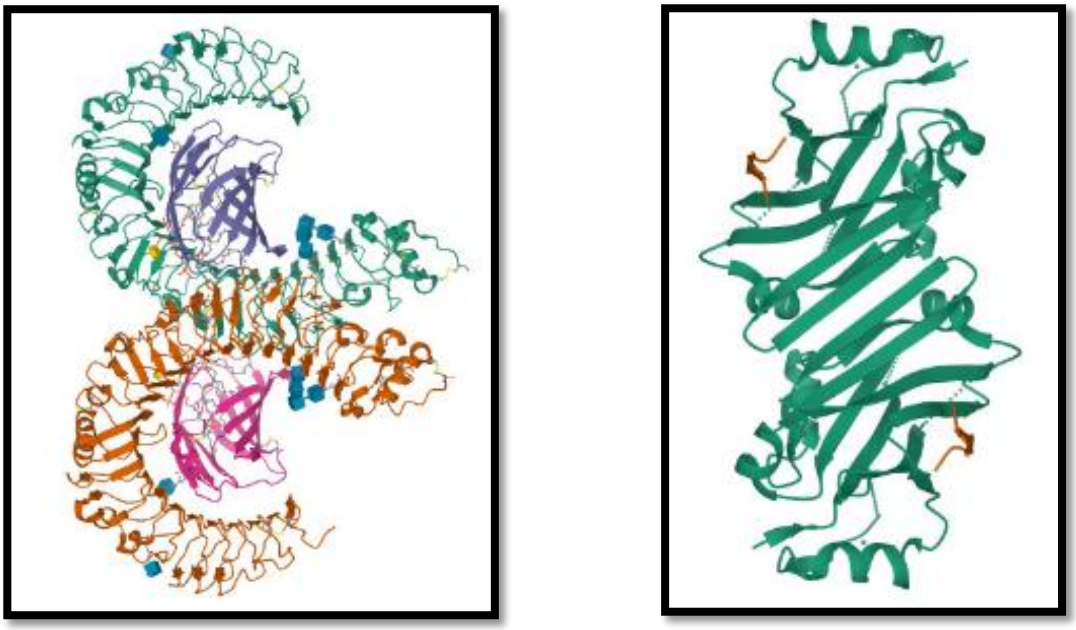
MATERIALS AND METHODS

Software- VLifeMDS

Tool- Pub Chem

Binding score evaluation by using software

The binding affinity score of lupeol and beta-sitosterol bioactive molecules on immunomodulatory receptors such as, Toll like receptor (4G8A), tumor necrosis factor (TNF-alpha) (PDB: 2AZ5) were evaluated, using VLifeMDS software. 3D chemical structures of bioactive molecules such as lupeol and beta-sitosterol were retrieved from Pub Chem. Thus, molecular docking studies were involved to estimate the binding affinity on immunomodulatory proteins.



A B

Fig.No.2. 3D Visualization of A- Toll like receptor, B- TNF-γ

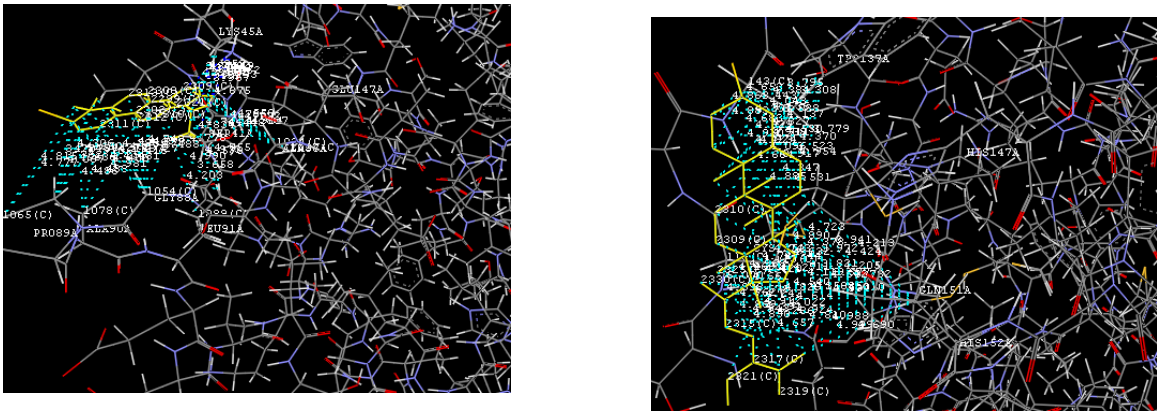
RESULTS AND DISCUSSION-

Since immunomodulation plays an important role in the formation of inflammatory mediators and regulation of innate and adaptive immune responses mainly increase transcription and expression of pro-inflammatory cytokines mediated by Toll like receptors and TNF-γ. The results of the molecular docking studies indicated that the interaction analysis suggested that lupeol having reactive groups take the form of hydrogen bonding with amino acids like SER41A, LYS45A, ALA86A, GLY88A, PRO89A, ALA90A, LEU91A in Toll like receptors with binding score -6.107 -K/Cal. Beta-sitosterol forms hydrogen bonding with amino acids such as CYS135A, TRP137A, GLN151A in TNF-γ with binding score -6.145.binding score was then compared with standard immunomodulatory agent levamisol having binding energy of -4.314 with TNF-γ and -4.934 with Toll like receptor. Docking results showed that lupeol and beta-sitosterol having binding energy and interactions with immunimodulatory receptors.

Table No.1. Binding affinity score of bioactive molecules on immunomodulatory receptor

Sr. No.	Name of the compound	Immunomodulatory receptor	Binding affinity (-K/Cal)
1	Lupeol	Toll like receptor (PDB:4G8A)	-6.107
2	Beta-sitosterol	TNF-γ (PDB: 2AZ5)	-6.145
3	Levamisol	TNF-γ (PDB: 2AZ5)	-4.314

4	Levamisol	Toll like receptor (PDB:4G8A)	-4.934



A B

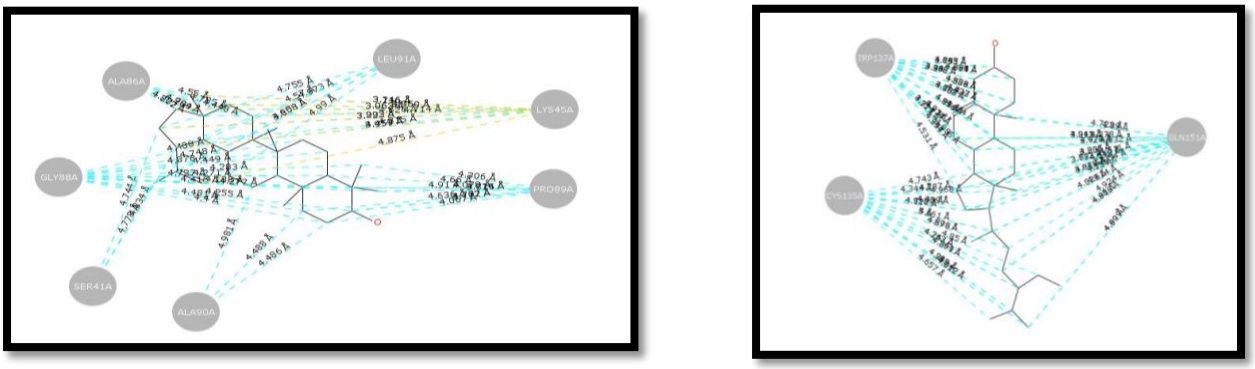
Fig.No.3. A-3D visualization of type of Interaction on Toll like receptor with lupeol, B- 3D visualization of type of Interaction on TNF-γ with Beta-sitosterol

Table No.2.Binding site interaction of *Delonix regia* phytochemical on human immunomodulatory receptors.

Sr. No.	Protein name	Interaction sites
1	Lupeol	**Interaction with Toll like receptor** H Bonding- SER41A, LYS45A, ALA86A, GLY88A, PRO89A, ALA90A, LEU91A Van der Waals- SER41A, LYS45A, GLY88A, PRO89A, LEU91A, GLU147A,
1	Beta-sitosterol	**Interaction with TNF-γ** H Bonding- CYS135A, TRP137A, GLN151A Van der Waals- CYS135A, TRP137A, HIS147A, GLN151A, HIS152A

CONCLUSION-

Bioactive molecules lupeol and beta-sitosterol having immunomdulatory potential from the results of molecular docking studies. So, further next approach is to isolate and identify this chemical phytoconstituents from various plant sources and use them for in-vitro and in-vivo immunomodulatory studies**.**



A B

Fig.No.4.

A- 3D interaction sites of proteins on Lupeol

B- 3D interaction sites of proteins on Beta-sitosterol

REFERENCES-

1. Nicholson LB. The immune system. Essays in biochemistry. 2016 Oct 31;60(3):275-301.
2. Kayser O, Masihi KN, Kiderlen AF. Natural products and synthetic compounds as immunomodulators. Expert review of anti-infective therapy. 2003 Jul 1;1(2):319-35.
3. Vetvicka V, Vetvickova J. Natural immunomodulators and their stimulation of immune reaction: true or false? Anticancer Research. 2014 May 1;34(5):2275-82.
4. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. Allergy, Asthma & Clinical Immunology. 2018 Sep;14(2):1-0.
5. Moresco EM, LaVine D, Beutler B. Toll-like receptors. Current Biology. 2011 Jul 12;21(13):R488-93.
6. https://en.wikipedia.org/wiki/Cytokine_receptor.
7. https://en.wikipedia.org/wiki/T-cell_receptor. Pasala PK, Reddy LS, Silvia N, Reddy YD, Sampath A, Dorababu N, Mulukuri NS, Kumar KS, Chandana MS, Chetty CM, Bendale AR. Molecular docking and in vivo immunomodulatory activity of Albizia procera bark on doxorubicin induced immunosuppressive rats. Journal of King Saud University-Science. 2022 Apr 1;34(3):101828.
8. Siddique HR, Saleem M. Beneficial health effects of lupeol triterpene: a review of preclinical studies. Life sciences. 2011 Feb 14;88(7-8):285-93.
9. Alappat L, Valerio M, Awad AB. Effect of vitamin D and β-sitosterol on immune function of macrophages. International Immunopharmacology. 2010 Nov 1;10(11):1390-6.