**Bulletin of Environment, Pharmacology and Life Sciences**
Bull. Env. Pharmacol. Life Sci., Vol 7 [11] October 2018 : 32-36

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95


**ORIGINAL ARTICLE** **OPEN ACCESS**

# Exploring immunomodulatory potential of Niaziminin B found in *Moringa oliefera* Lam. In chicken through *in silico* modeling and Molecular docking studies

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**ABSTRACT**
*Moringa oleifera (drum stick) of family Moringaceae is one of the best known medicinal plants. The vital minerals present in Moringa oleifera include Calcium, Copper, Iron, Potassium, Magnesium, Manganese and Zinc. Moringa oleifera leaves, pods, seeds, gums, bark and flowers possess medicinal potential and its usage leads to gain a healthy cardiovascular system, promote normal blood-glucose levels, neutralize free radicals, provide excellent support of the body's anti-inflammatory mechanisms, enrich anemic blood and support immune system. Around the globe poultry industry has been growing persistently. Many reports have shown that inclusion of herbs in poultry diet can lead to improved health condition of birds. Cytokines are the glycoproteins that play crucial role in modulation of immune response. Herbal plants exhibit their therapeutic potential due to the presence of various phytocontituents that may cause immunomodulation by directly interacting with cytokines. Thus the present study was carried out to explore role of an important phytoconstituent niaziminin B of Moringa olifera for its immunomodulatory potential through conducting in silico docking studies with various cytokines, viz., IL-1β, IL-2, IL-4, IL-5, IL-10 and Interferon gamma of Gallus gallus (chicken). The protein sequence of interleukin genes were retrieved from NCBI and 3D structures were predicted through Swiss Model tool. The 3D structure of niaziminin B was retrieved from pubchem. Molecular docking studies were performed by using PatchDock server between the phytocompound and chosen receptors and then these results were analyzed. It was observed that niaziminin B exhibited significant binding affinity with IL-5, IL-1β, IL-4 and IL-2. Thus it could be inferred from the present study that interaction of niaziminin B with various cytokines play important role in immunomodulatory potential of Moringa oleifera.*
***Keywords:*** *Immunomodulation; Moringa oleifera; Niaziminin B; Cytokines; Gallus gallus; molecular docking.*

Received 21.06.2018 Revised 22.08.2018 Accepted 23.09.2018

## INTRODUCTION

Many plant based products have been used for immunomodulation since long in old civilizations. Environmental stresses and nutritional status affects the immune system and it is advocated that diet rich in antioxidants and micronutrients can boost the immune system [1-3]. *Moringa oleifera*Lam. is widely used in folklore for the treatment of suppressive conditions of immune system. Several studies showed the stimulatory actions of *Moringa oleifera* on immune system [17]. *Moringa oleifera* is a medicinal plant and an excellent dietary source of micronutrients (vitamins and minerals) and health-promoting phytochemicals (phenolic compounds, glucosinolates and isothiocyanates). Glucosinolates and isothiocyanates are known to possess anti-carcinogenic and antioxidant effects. Three known thiocarbamate (TC) and isothiocyanate (ITC) related compounds have been isolated from the leaves of *Moringa oleifera*, a traditional herb in Southeast Asia [18, 19, 6, 7]. The main constituents of *Moringa oleifera* include two nitrile glycerides: niazirin and niazirinin and three mustard oil glycosides isothiocynate, niaziminin A and niazimin B.The mustard oil glycosides were attributed for hypotensive activity of *Moringa oleifera. Moringa oleifera* is reported to possess antioxidant with immunostimulatant property as per traditional knowledge [17, 9-12].

Cytokines are responsible for crosstalk between cells of immune system and are mediators of immune response. These are small protein molecules produced by a number of cell types, predominantly

leukocytes that regulate immunity, inflammation and hematopoiesis. These molecules can exhibit synergistic, antagonistic and pleiotropic activities. IL-1β plays an important role in both innate and adaptive immunity and is a crucial mediator of the host inflammatory response in natural immunity [16]. IL-2 has essential roles in key functions of the immune system, tolerance and immunity, primarily via its direct effects on T cells. IL-2 also promotes the differentiation of T cells into effector T cells and into memory T cells when the initial T cell is also stimulated by an antigen, thus helping the body fight off infections [15]. Interleukin-4 has many biological roles, including the stimulation of activated B-cell and T-cell proliferation and the differentiation of B cells into plasma cells. It is a key regulator in humoral and adaptive immunity. IL-4 induces B-cell class switching to IgE, and up-regulates MHC class II production. IL-4 decreases the production of Th1 cells, macrophages, IFN-gamma, and dendritic cell IL-12 [13]. Interleukin 5 stimulates B cell growth and increases immunoglobulin secretion - primarily IgA. It is also a key mediator in eosinophil activation. IL-10 is a cytokine with multiple, pleiotropic, effects in immunoregulation and inflammation. It also enhances B cell survival, proliferation and antibody production. IL-10 can block NF-κB activity, and is involved in the regulation of the JAK-STAT signaling pathway.IFN-γ, or type II interferon, is a cytokine that is critical for innate and adaptive immunity against viral, some bacterial and protozoal infections. IFN-γ is an important activator of macrophages and inducer of Class II major histocompatibility complex (MHC) molecule expression [20-22].

Niaziminins A and B have previously been obtained from the leaf extract as a mixture. Niaziminin A and B showed hypotensive activity while nitrile glycosides 1 and 2 were found to be inactive [8]. Leaves of *Moringa oleifera* are reported to possess antioxidant, immunomodulatory and hepatoprotective potential [18, 19, 11, 4]. Keeping in view of above present *in silico* analysis was carried out to explore immunomodulatory potential of niaziminin B of *Moringa oleifera* in chicken by conducting *in silico* molecular interaction studies with different cytokines *viz*., IL-1β, IL-2, IL-4, IL-5, IL-10 and Interferon gamma of *Gallus gallus*.

## MATERIAL AND METHODS

### Ligand Preparation

Present study included one ligand whose three-dimensional (3D) structure was retrieved from Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) in sdf format, these files cannot be directly used by Patchdock, thus they were converted into pdb format by using Open Babel, a software package used to change the format of the input file for further studies (Table1).

### Receptors Preparation

The FASTA format used in the present study were retrieved from the NCBI data bank. The FASTA sequence converted to 3D structure by SWISS Model, an online server. The raw PDB structure should be prepared in a suitable manner for molecular docking studies. AutoDock Tools was used to prepare the protein structure. During protein structure preparation, water molecules and peptide substrate were deleted while hydrogen atoms for generation of final 3D structures of respective compound were added.

### Automated Molecular Docking

The ligand-receptor interactions were studied using PatchDock server (http://bioinfo3d. cs.tau.ac.il /PatchDock/). The ligand niaziminin B was docked with all six studied receptors on geometry based algorithm. After the fast transformational search, the best geometric fit obtained the highest scores, while the low scores exhibited poor matches.

### Analysis of docked complexes

The binding patterns of docked complexes thus obtained were analyzed on PyMol molecular viewer (http://www.pymol.org/) and the docked positions were captured in the form of pictures in each case. The interacting residues of cytokines and interacting molecules of the ligands were labeled. The hydrogen bond lengths were also labeled.

## RESULTS AND DISCUSSION

The molecular docking experiments revealed the binding and interaction patterns of niaziminin B with all studied receptors. The results were obtained in the form of scores and atomic contact energy(ACE) of the docked complexes (Table 2, Figure 1). Patchdock Online server is used to find out the binding mode and ACE between proteins and the phytocompound. The binding affinity of niaziminin B is presented in the docking score which is 5728 for IL-1β, 5062 for Interferon gamma, 4972 for IL-10, 4914 for IL-2, 4722 for IL-5 and 4630 for IL-4. Figure 2 indicates the interaction of niaziminin B with all studied cytokines.

There are several reports available showing immunomodulatory potential of leaves of *Moringa oleifera*. The extract of *Moringa oleifera* leaves inhibited human macrophage cytokine production of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and IL-8), which were induced by cigarette smoke and by lipopolysaccharide (LPS) [14]. Further, Waterman *et al*. [24] reported that *Moringa oleifera* decreased

the gene expression and production of inflammatory markers in RAW macrophages. The *Moringa oleifera* leaves extracts stimulated both cellular and humoral immune responses in cyclophosphamide-induced immunodeficient mice, through increases in white blood cells, percent of neutrophils and serum immunoglobulins [23, 11]. In the present study, niaziminin B, a phytoconstituent of leaves of *Moringa oleifera, indicated active involvement in* immunostimulation through the *in silico* molecular docking analysis with various chicken cytokines.

Table 1- Properties and three-dimensional (3D) structure of Niaziminin B

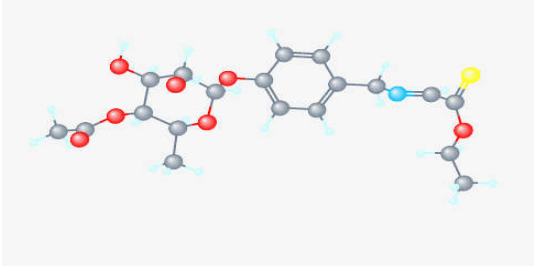
| | |
|---|---|
| **Compound name:** | Niaziminin B |
| **PubChem CID:** | 44559760 |
| **Molecular Formula:** | $C_{19}H_{25}NO_7S$ |
| **Molecular Weight:** | 411.469 g/mol |
| **3D Structure:** |  |
| **Hydrogen Bond Dnor Count:** | 2 |
| **Hydrogen Bond Acceptor Count:** | 9 |
| **XLogP3-AA:** | 1.7 |

Table 2- The scores and ACE (atomic contact energy) of docked complexes.

| Receptor | NiazimininB | |
|---|---|---|
| | Score | ACE |
| Interleukin-1 β | 5728 | -268.55 |
| Interleukin-2 | 4914 | -207.31 |
| Interleukin-4 | 4630 | -236.67 |
| Interleukin-5 | 4722 | -270.44 |
| Interleukin-10 | 4972 | -163.92 |
| Interferon gamma protein | 5062 | -175.37 |

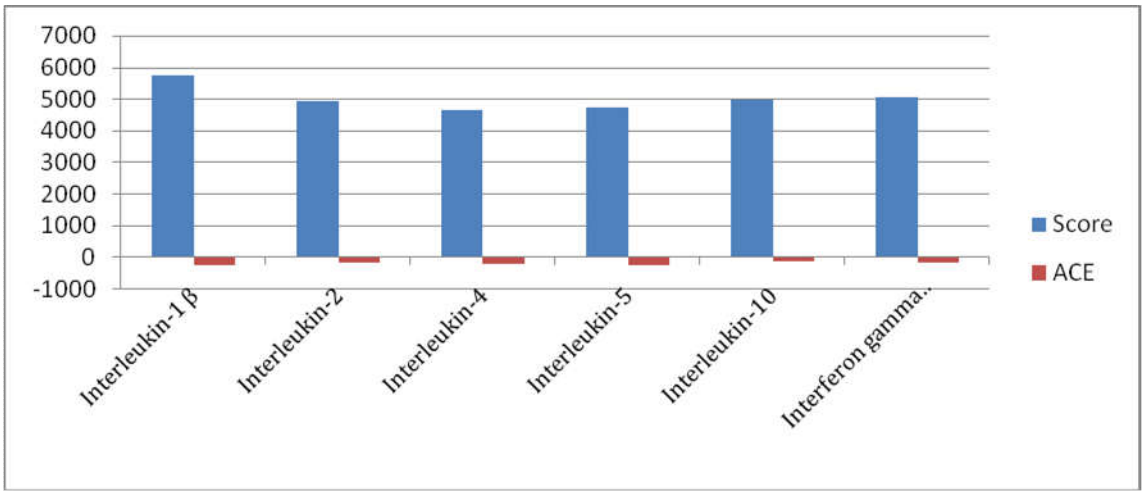



Fig.1 – Graph representing docking scores and ACE of Niaziminin B with all cytokines

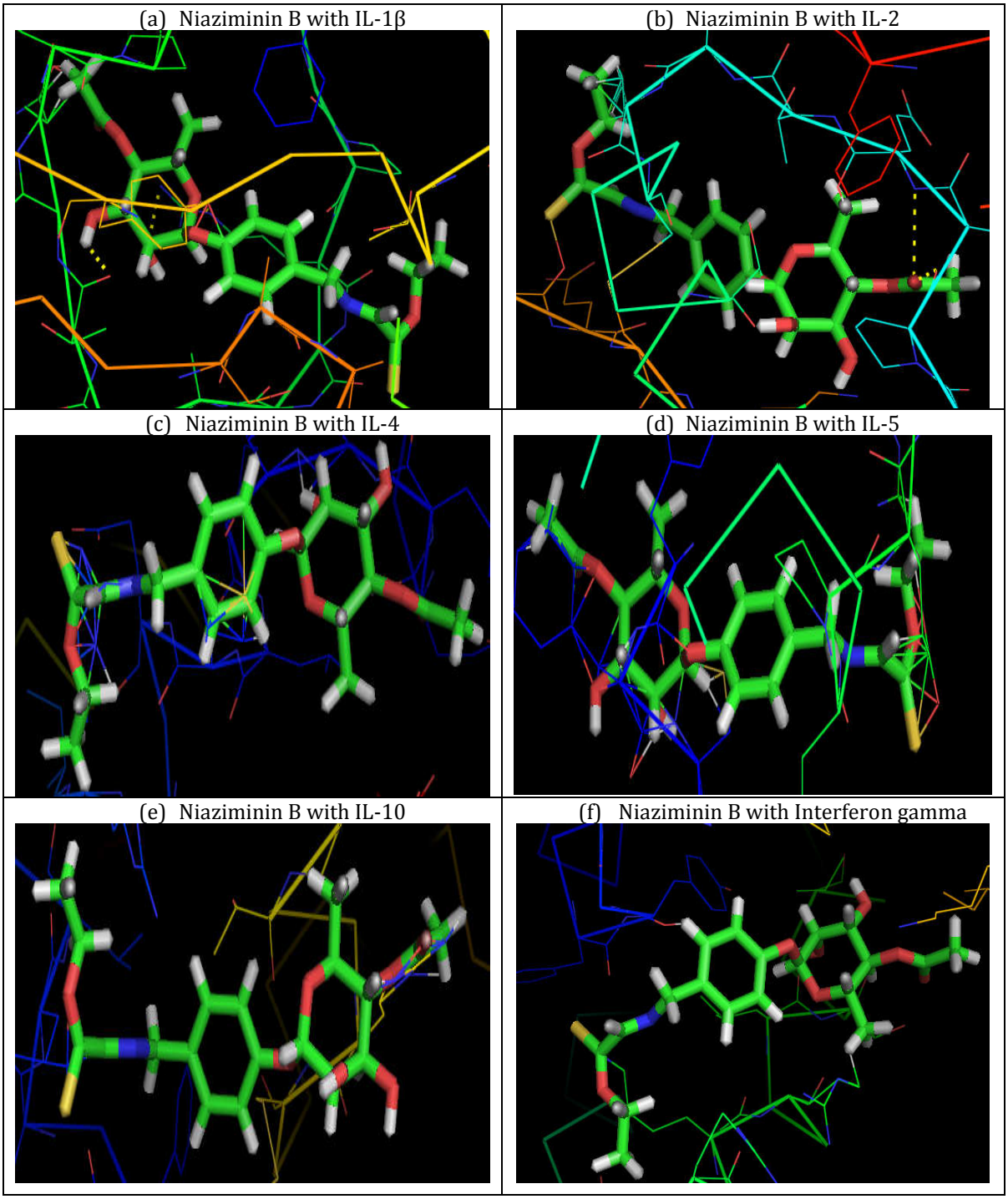



Fig.2- Molecular docking interactions of Niaziminin B with various chicken cytokines

## CONCLUSION

Niaziminin B showed significant interaction with IL-1β and Interferon gamma followed by other cytokines which signifies its active role in immunomdulatory potential of *Moringa oleifera*. Thus, through *in silico* studies it was predicted that niaziminin B could be responsible for immunomodulatory potential of *Moringa oleifera* through cytokines mediated signaling pathways which could be further confirmed through suitable *in vitro*/ *in vivo* experimentations.

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**CITATION OF THIS ARTICLE**

Mayuri Agarwal and Sonu Ambwani. Exploring immunomodulatory potential of Niaziminin B found in *Moringa oliefera* Lam. In chicken through *in silico* modeling and Molecular docking studies . Bull. Env. Pharmacol. Life Sci., Vol 7 [11] October 2018: 32-36