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In-Silico Analysis for Predicting the Potential of Constituents from *Nigella sativa* as an anti-Inflammatory Drugs Candidate for SLE

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Abstract— *Nigella sativa L.* belongs to family *Ranunculaceae*. The constituent from the seeds of *Nigella Sativa* has been reported to possess antitumor, antioxidant, antibacterial, anti-inflammatory, hypoglycemic, central nervous system depressant, antioxidant, and immunomodulatory activities. Systemic Lupus Erythematosis (SLE) is a multisystemic inflammatory disease due to chronic autoimmune disease. Current therapy has not shown satisfactory results due to various side effects. **Objective**:Topredict the potential of constituents from *Nigella sativa* as an anti-inflammatory drug and as a SLE novel therapy. **Method**: The compound contained in Nigella was obtained from KnapackKaraya web server. *Nigella sativa*'s potential as an anti-inflammatory is predicted using Pass Server and Way2Drug. The parameters analyzed were updated agonist factor macrophage colonies, Caspase 3 stimulants, Caspase 8 stimulants, TP53 expression enhancers, FABP5, apoptotic agoptics, and immunomodulators. Interaction Experiment Tool with Protein Interest, Search Tool 17 Interacting Chemicals (STITCH). Target protein prediction (HITPICK).**Result**: Pass Server Analysis shows that *Nigella sativa* can act as an anti-inflammatory and induce apoptosis through TP53 expression enhancer and Caspase stimulant (Pa > 0.3). STITCH analysis shows that the content of *Nigella sativa*, Carvacrol, Thymol, and Myristicin, are predicted to activate CASP3 (Caspase 3) to trigger apoptosis. Analysis of HITPICK Oleic acid compounds is predicted to target FABP5 with 100% precision which can activate IL17A related to cell inflammation and autoimmune process.**Conclusion**: Based on an in-silico study, it is revealed that constituent metabolites from *Nigella sativa* seeds were predicted to have potential as anti-inflammatory agents.

Keywords: Autoimmune,*Nigella sativa,* in silico, SLE, anti-inflammatory

1. Introduction

Systemic Lupus Erythematosis (SLE) is a multisystemic inflammatory disease due to chronic autoimmune which has very diverse clinical manifestations, pathogenesis and prognosis. SLE is more common in women of productive age (14–64 years) compared to men, with a ratio of 9–15 to 1, and women of Black race have the highest risk of developing SLE followed by Asian women and white women, and it has been shown that genetic, immunological, hormonal, and environmental factors play an important role in the pathophysiology of SLE. [1]. In children, the incidence of SLE varies between 0.36–2.5 per 100,000 population per year with a prevalence between 1.89–25.7 per 100,000 population. [1,2,3,4,5] In Indonesia, based on Infodatin 2017, it is estimated that the number of SLE patients in Indonesia reaches 1,250,000 people. [6] Mortality and morbidity rate of SLE are quite high, due to the accumulation of tissue damage caused by disease activity. Mortality is generally caused by infection in the first few years and cardiovascular disease in the long term. [2,5]

In Indonesia, the diagnosis of SLE is based on the criteria from the *American College of Rheumatology* in 1997 which has been revised and validated by *The Systemic Lupus International Collaborating Clinics* (SLICC) which involve clinical manifestations and immunological response in

patients.[2] Clinical manifestations of SLE include the involvement of skin, mucosa, joints, blood, heart, lung, kidney, central nervous system, and immune system. The immunological criteria are based on the results of ANA and anti-dsDNA tests. [1,3,4,5,6]

Although, until now,there is no therapy that can cure SLE; therapy is only intended to cause remission of disease symptoms, prevent further damage, minimize the effects of drugs, and improve the quality of life of patients, SLE therapy is drugs which are in the form of non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease modifying drugs which are administered according to the severity of the disease. [1,2,4-6]The side effects of drugs are one of the most common causes of death in SLE as well as other causes including cardiovascular disease, infections, and active SLE. [7] The long term use of lupus drugs has quite severe side effects, especially in children. Therefore, at this time a natural-based drug as an affordable anti-inflammatory and immunomodulatory with minimal side effects is needed to reduce symptoms and inflammation in lupus.

Several herbal therapies have been investigated and proven in their ability to improve SLE symptoms. Where*Nigella sativa* with the active constituent is one of the herbs that is proven to reduce inflammation and modulate the immune response in several autoimmune diseases, [8] herbs can suppress or improve several aspects of the immune system, so they are called anti-inflammatory with minimal side effects. However,there is still little research on the use of *Nigella sativa* in lupus sufferers. This study aims to analyze the potential of *Nigella sativa* as an anti-inflammatory with the in-silico model based on the bioinformatics approach.

2. Method

2.1 Target selection

Pass Server. Pass server is a structural approach; the server will compare the compounds entered with compounds that have been shown to have certain activities. WherePa (probability to be active) is a value that describes the ability of compounds in certain biological processes, the higher the Pa value, the more similar the structure and function of the compound and the more potential it has. If Pa has a value of more than 0.7, the similarity of the input compound to the database is high, so it is probable that the compound has biological activity if tested in a laboratory. If the Pa value is more than 0.3, but less than 0.7, the compound has potential as a computational bioactivity because there is little similarity in structure with the compound recorded in the database, and further laboratory testing is needed. [10].***ay2drug*** is theweb server to predict the bioinformatics potential of the compound contained in *Nigella sativa,* which is obtained from the KnapSackKanaya web server, then the compound is predicted to have potential as an immunomodulator.

2.2 Interaction between molecules

STITCH is used forintegrated information about the interactions of the metabolic pathway, crystal structure, experiments about binding, and target drug relationships. [9] Analysis using Stitch, compounds in *Nigella sativa* which are Carvacrol, Thymol, and Myristicin are predicted to interact with CASP3 (Caspase 3).***HITPICK*** is used to predict proteins targeted by the compound content in EVOO (Extra Virgin Olive Oil)**.** Salah satusenyawa Nigella sativa, Oleic acid is predicted to target FABP5 with 100% precision.

3. Results And Discussion

3.1 *Way2drug Pass*

The compound contained in *Nigella sativa* was obtained from the KnapSackKanayawebserver, then the potential of the compound was predicted as an immunomodulator. The potential of *Nigella sativa*

as an immunomodulator is predicted using Pass Server. There are several parameters analyzed, including the following: Macrophage colony stimulating factor agonist; Caspase 3 stimulant; Caspase 8 stimulant; TP53 expression enhancer; Apoptosis agonist Immunomodulator

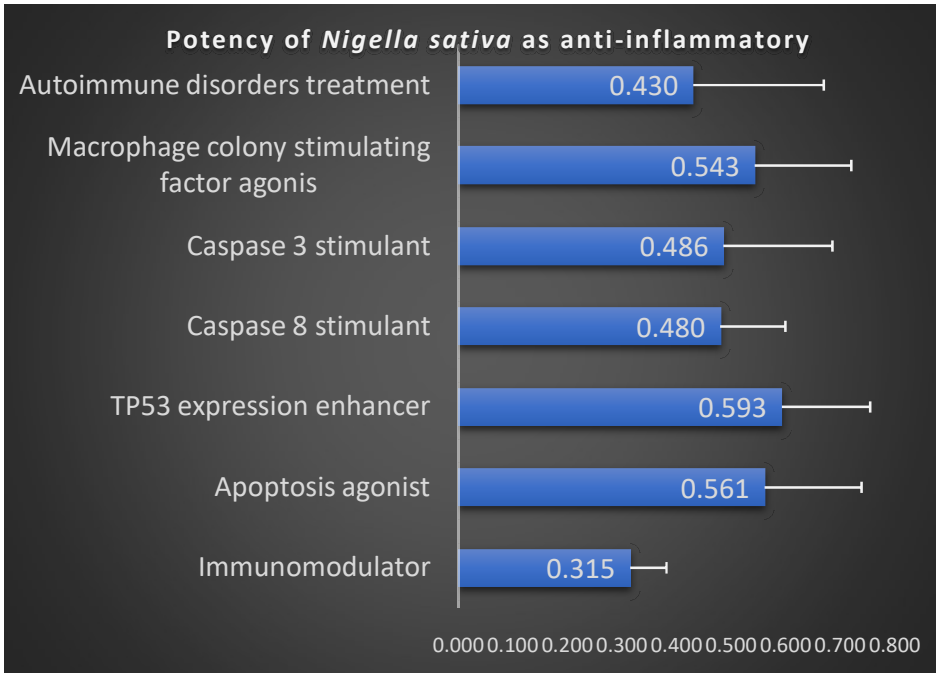


Figure 1 Pass analysis on *Nigella sativa*

Based on the analysis using Pass Server, *Nigella sativa* is predicted to be used for the treatment of immune diseases. As shown in Fig. 1, *Nigella sativa* can act as a macrophage colony stimulating factor (Pa>0,5), immunomodulator (Pa>0,3) and induce apoptosis (Pa>0,5) through TP53 expression enhancer (Pa>0,5) and Caspase stimulant (Pa>0,4). Activation of macrophage colony stimulating factor have a protective role for autoimmune disease. The protective effect of GM-CSF was associated with a selective expansion of CD11c+CD8a− DCs. They also observed a reduction in anti-AChRAb levels, T cell propagation, and Th1 cytokine responses, and an increase in the IL-10 response. This effect was likely due to a shift in the cytokine milieu to a Th2 profile and the generation of Tregs13 .

Gaudreauet al. have found that treatment of NOD mice with GM-CSF can protect them from diabetes and increase the number of splenic CD11c+CD11b+CD8a− DCs. That protection was possibly associated with the accumulation of tolerogenic immature splenic DCs and Tregs. Also, GM-CSF promotes the development of semi-mature DCs that recruit Th2 and Tr1 cells and inhibit diabetes in NOD mice as well as autoimmune thyroiditis (98).ffect of TP53 on the promoter; TP53 enhances Foxp3 expression through the activation of CNS-2 presumably by acting cooperatively with transcription factors such as Foxp3, Runx1, Cbf-β, CREB/ATF, and STAT5. TP53 directly activated the promoter and the CNS2 of *Foxp3* gene, which is a Tregactivation pathway14.

3.2 Prediction of Interaction of Compounds with Protein Interest (STITCH)

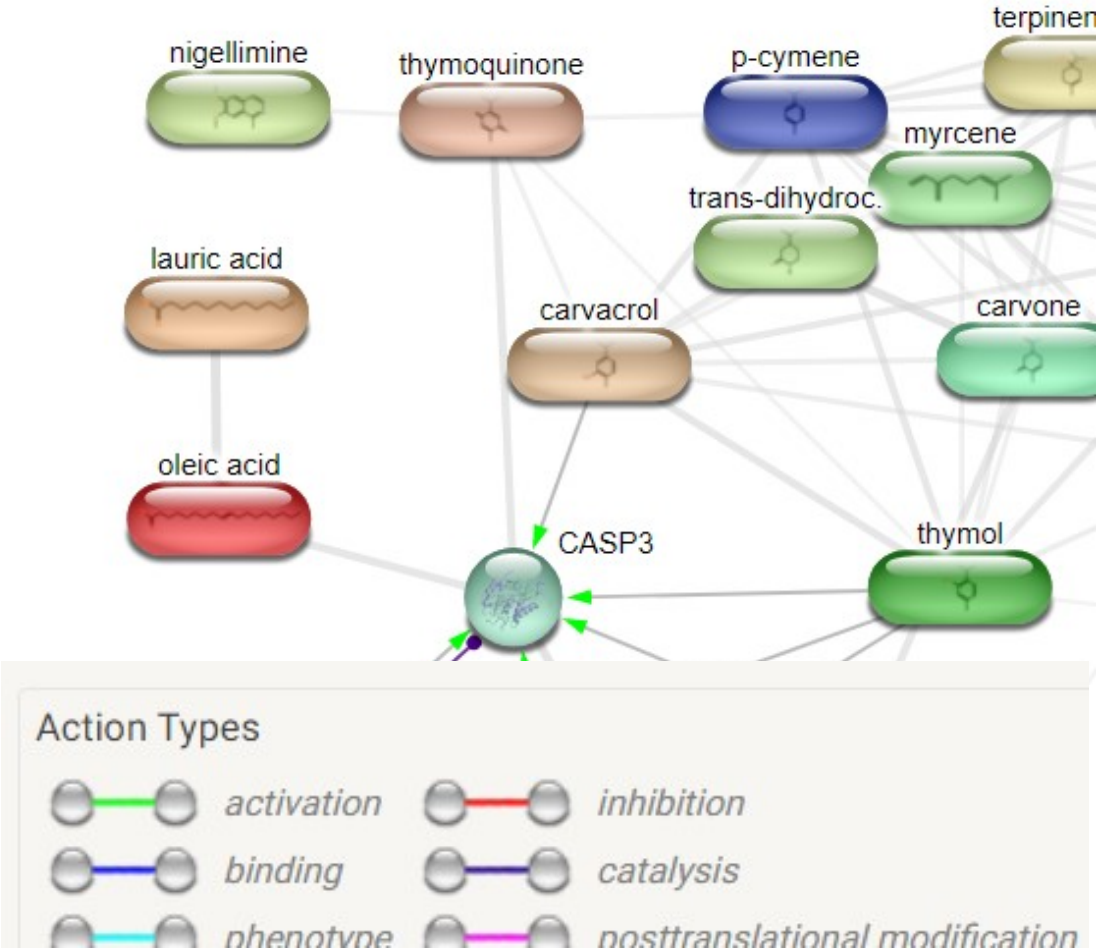


Figure 2 Predictions of compound interactions in *Nigella sativa*

Based on analysis using Stitch, the constituents in *Nigella sativa* such as Carvacrol, Thymol, and Myristicin are predicted to be able to interact with CASP3 (Caspase 3), CASP8 (Caspase 8),and CASP9 (Caspase 9) where the interaction that occurs is activation. CASP3 is a protein needed for cells to carry out apoptosis. Inducing CASP3, CASP8, and CASP9 activation can quickly eliminate autoreactive T cells via apoptosis and inhibit autoimmune disease.

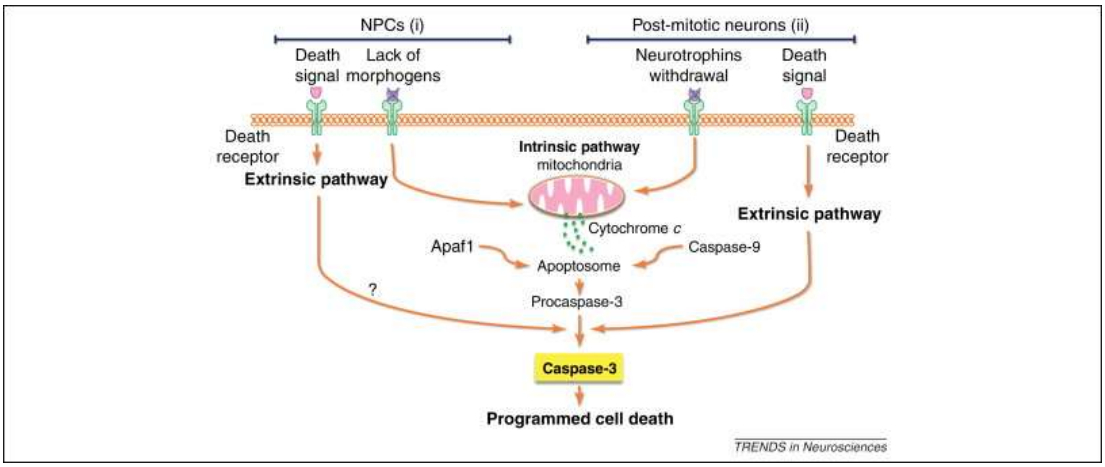
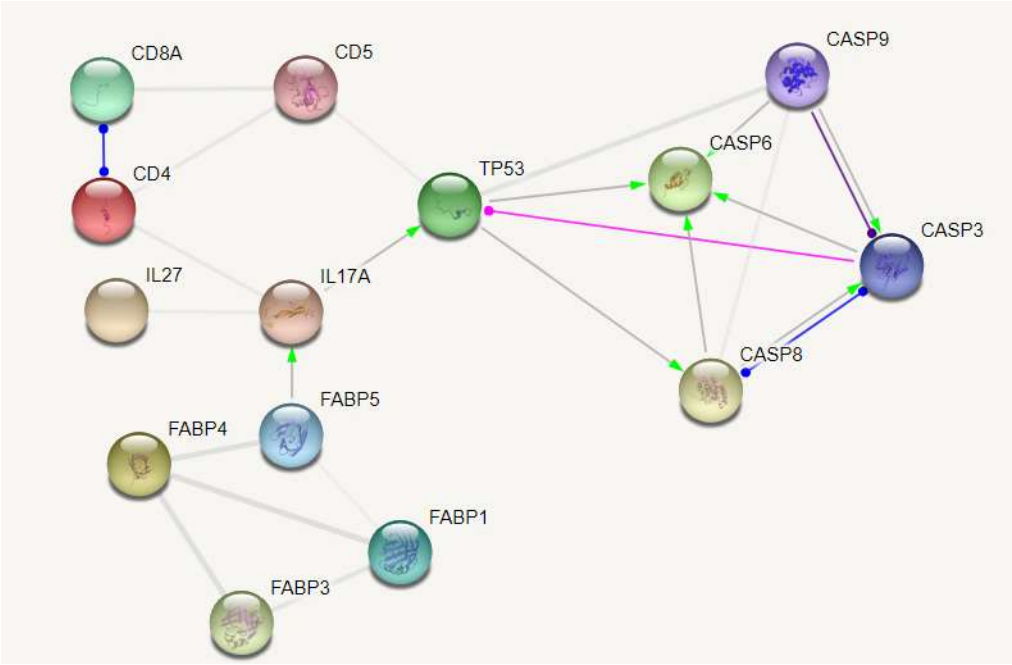


Figure 3 Cell apoptosis pathway 21

Caspase-3 and the regulation of programmed cell death (PCD). Caspase-3 regulates PCD in neural precursor cells (NPCs) and post-mitotic neurons16,17.NPCs undergo apoptosis by apoptosome formation on the withdrawal of morphogens, or potentially on signaling via death receptors 17,18. By contrast, the absence of neurotrophins and the consequent lack of stimulation of its receptor induce PCD in post-mitotic neurons 17. The action of the extrinsic death receptor pathway has been described in post-mitotic neurons; by contrast, this role has not clearly been elucidated in NPCs. Where activation of the intrinsic cell death pathway results in induction of cytochrome *c* release from mitochondria, which together with Caspase 9 and Apaf1 constitutes the apoptosom,15 this activation is caused by lack of morphogens in NPCs or neurotrophin withdrawal in post-mitotic neurons. Activation of this pathways results in induction of cytochrome *c* release from mitochondria, which together with Caspase-9 and Apaf1 constitutes the apoptosom,15 which is a catalytic multiprotein platform responsible for converting Procaspase 3 into active Caspase 316, which subsequently orchestrates the dismantling of cell structure.

3.3 Prediction of Target Protein Interaction with Interest Protein (HITPICK)



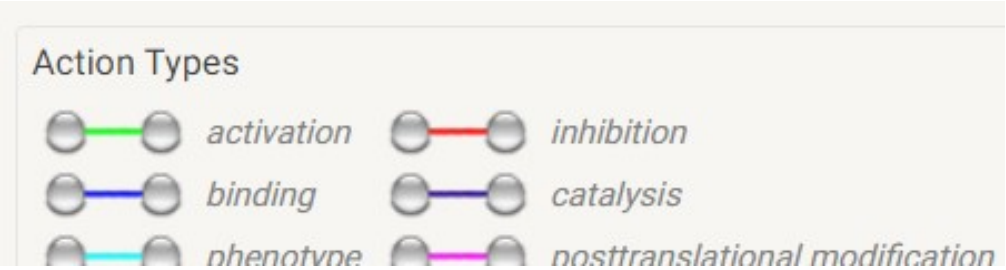


Figure 4 HITPICK protein in *Nigella sativa*

As shown in Fig. 4, FABP5 is predicted to activate IL17A. Il17 was responsible for the development of inflammation in many disorders, especially in autoimmune diseases such as rheumatoid arthritis (RA), psoriasis, juvenile idiopathic arthritis (JIA), Crohn's disease and SLE11. Field et al. show that fatty acid-binding protein 5 (FABP5) maintains mitochondrial integrity in regulatory T cells (Tregs). FABP5 inhibition results in mtDNA release, which triggers expression of IL-10 and promotes Treg suppressive capacity. These findings may have implications for therapeutically targeting Tregs in autoimmunity and cancer.20

FABP5 is a target protein from Oleic acid obtained through HitPick Target Prediction. Hitpick is used to predict proteins targeted by the compound content in EVOO. HITPICK will compare the query structure (input compound) with the database that is already available. The highest precision is 100%. Not all compounds in EVOO have interactions with proteins that play a role in the immune system. Oleic acid is predicted to target FABP5 with 100% precision. FABP5 (Fatty Acid-Binding Protein 5) is a protein that can modulate inflammation by regulation of PTGES by induction of NF-kappa-B activation, and prostaglandin E2 (PGE2) biosynthesis during inflammation.

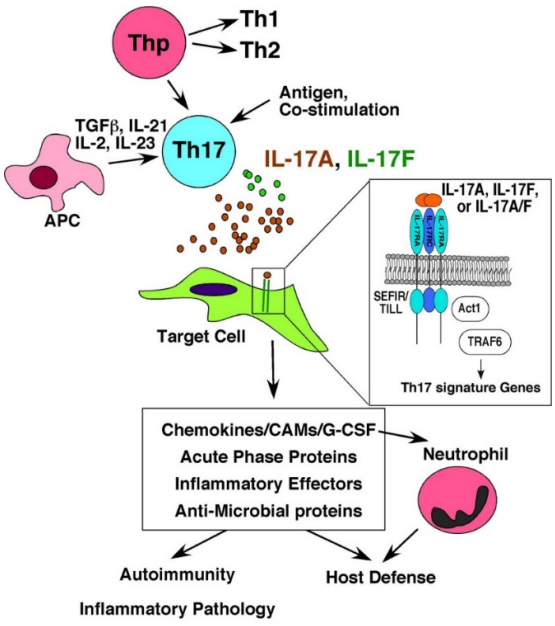


Figure 5 Role of Th17 in the Inflammation Process 23

There are six known IL-17 isoforms, from A to F, but Th17 cells are only able to produce IL-17A and IL-17F. Both are proinflammatory cytokines. Some researchers have recently shown that IL-17A and / or IL-17F are responsible for the development of inflammation in many disorders, especially in

autoimmune diseases such as rheumatoid arthritis (RA), psoriasis, juvenile idiopathic arthritis (JIA), Crohn's disease, and many others. [11]

Interaction between N. sativa protein targets and TH17 and T regulators

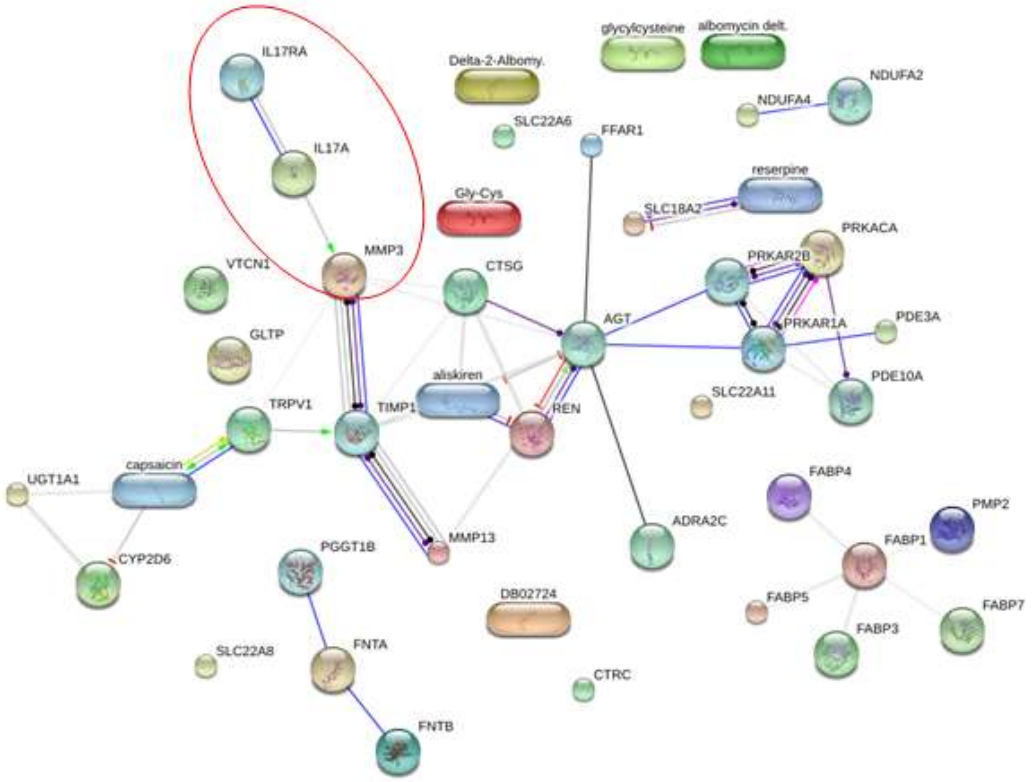


Figure 6 STITCH Analysis IL 17

To analyze the interaction of the target protein and its relationship with the TH17 and T regulators, a network analysis was performed using the STITCH program. Because Th17 input is not recognized by the system on STITCH, IL17 is used, the production of which is characterized by the presence of Th17 cells as CD4 effectors (+).The results show that IL17 activates MMP3 (indicated by a green arrow) which is one of the target proteins that will be inhibited by the compound N. Sativa, namely anisaldehyde. MMP3 can degrade fibronectin, laminin, gelatins type I, III, IV, and V; collagens III, IV, X, and IX, and proteoglycan cartilage, as well as activating procollagenase.By decreasing the ability to uptake DA and increasing LDH expenditure in Parkinson's disease, MMP3 can suppress cellular phenomena that occur in DArgic neuron cells that die. MMP-3 deficiency can reduce the ability to uptake DA. In addition, decreased MMP-3 expression with siRNA is able to protect cells. MMP3 is able to cut Procaspase 3 indirect, through the activation of several proteases. Overall, MMp3 has an important role in apoptotic signaling after the intracellular process of proMMP3 to act asMMP3 under stress conditions. MMP3 can potentially be a target of cellular antagonists in neuroprotective therapy[21].

4. Conclusion

Based on the analysis using Pass Server,*Nigella sativa* can act as an immunomodulator and induce apoptosis through TP53 expression enhancer and Caspase stimulant (Pa>0.3). STICH analysis shows that the compositions of *Nigella sativa,* which are Carvacrol, Thymol, and Myristicin, are predicted to

be used with CASP3 (Caspase 3) for inducing apoptosis. From the HITPICK test, the Oleic acid is predicted to target FABP5 with 100% precision. So, based on *in-silico* studies, it was revealed that constituent metabolites from *Nigella sativa* seeds were predicted to have potential as anti-inflamasi and can be novel drug therapies for autoimmune disease Investigations in this line of study *invitro* and *in vivo* merits further evaluation.

Acknowledgement
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Apoptosis figure 3

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Figure 3

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