INNOVARE JOURNAL OF MEDICAL SCIENCES

Vol 11, Issue 2, 2023

ISSN - 2321-4406

Research Article

EXPLORING THE PHARMACOLOGICAL MECHANISM OF NARINGENIN AND ITS DERIVATIVES AGAINST RHEUMATOID ARTHRITIS USING NETWORK PHARMACOLOGY AND MOLECULAR DOCKING TECHNIQUES

VINITHA JANICE MONTEIRO*

Department of Bioinformatics, BioNome, Bengaluru, Karnataka, India. Email: janicevinitha@gmail.com

Received: 30 January 2023, Revised and Accepted: 15 February 2023

ABSTRACT

Objective: Flavonoids like Naringenin (NR), which have potent anti-inflammatory effects, are crucial in the treatment of rheumatoid arthritis (RA). Using network pharmacology and molecular docking, this study investigates the potential pharmacological mechanism of NR and its derivatives in the treatment of RA.

Methods: The current study's purpose was to employ computational methodologies to evaluate the efficiency of several NR phytochemicals against RA. The Indian medicinal plants, Phytochemistry and therapeutics and DrugBank database is used to retrieve potential ligands. While, known target proteins associated with RA were retrieved through the GeneCards database and predicted target proteins related to NR were screened through the STITCH database. STRING database was used to construct a protein-protein interaction network. Gene ontology and Kyoto Encyclopaedia of Genes and Genomes pathway enrichment involved in targets were performed by the ShinyGo 0.76.3 database. The BIOVIA Discovery Studio Visualizer and the virtual screening tool PyRx were used to systematically perform molecular docking. To assess their compatibility with the RA, the top 6 phytocompounds from NR were selected. The pharmacological evaluation of the ligands was carried out using ADMET filters.

Results: The phytocompounds 4'-Hydroxyflavanone and Sakuranetin from the NR derivatives were discovered to be the most potent antagonistic for the protein tumor protein P53 and interleukin-10 protein.

Conclusion: Ligands 4'-Hydroxyflavanone and Sakuranetin are deserving candidates for the suppression of inflammation of RA due to their strong affinity for the protein.

Keywords: Rheumatoid arthritis, Naringenin, Autoimmune, Molecular docking, Network pharmacology.

 DOI: http://dx.doi.org/10.22159/ijms.2023v11i2.47144. Journal homepage: https://innovareacademics.in/journals/index.php/ijms

INTRODUCTION

The medicinal herb is a major source of bioactive compounds with a variety of pharmacological activities. Among these, polyphenols are a hugely diverse category of substances widely present in plants and compared to synthetic drugs, they are more readily available, more tolerable and have fewer negative effects [1]. Naringenin (NR) (5, 7, 4-trihydroxyflavanone) is a flavanone aglycone that is a member of the large family of polyphenols known as flavonoid compounds. Inactive naringin that occurs naturally is transformed into active NR by bacteria gut microbiome. It is abundant in a variety of citrus fruits (grapefruit, limes, pomelos, oranges, and lemons), bergamot, tomatoes, and other fruits and vegetables [2]. Growing evidence from both *in vitro* and *in vivo* animal studies has supported NR's pharmacological effects as a hepatoprotective, anti-atherogenic, anti-inflammatory, anti-mutagenic, anticancer, and antimicrobial agent, even pointing to its potential use in the management and control of cardiovascular, gastrointestinal (GI), neurological, metabolic, infectious, and malignant diseases [3].

Rheumatoid arthritis (RA) is the most prevalent chronic inflammatory and auto-immune condition which means the immune system attacks healthy tissue and which mainly affects the wrist, fingers, and knee joints [4,5]. However, its origin is not yet known, which manifests as inflammatory alterations in the synovial joint tissue, cartilage, and bone, less commonly in extra-articular locations [6]. If it is not treated on time, it can result in painful joint damage and weakens the joint deformities. The symptoms of RA illness frequently include pain, exhaustion, swelling, warmth and redness, and stiffness of the joints [7]. It has been noted that RA carries a significantly increased risk for cardiovascular disease. Strong evidence suggests that increasing physical activity and/or exercise may simultaneously help RA symptoms and lower the severity of systemic signs [8].

RA affects up to 1% of the world's population and can affect anybody, regardless of color, sex, ethnicity, nationality, age, etc., the prevalence of RA is greater in women than in men which can cause significant joint damage and disability [9-12]. Statistics show that women have a 3.6% chance of acquiring this condition whereas 1.7% risk for males [13]. According to epidemiological research, the greatest prevalence ratios were seen in Japan (1.7%) and Argentina (1.97%), and the greatest incidence rate was found in the UK (27.5 cases per 100,000 inhabitants) [12]. Although the specific cause of RA is not known, it is likely caused by genetics, smoking, obesity, infection, periodontal disease, and the microbiota of the gut [14]. The pathophysiology of RA is thought to involve autoimmunity, which is characterized by the existence of distinctive autoantibodies such as rheumatoid factor or anti-citrullinated peptide autoantibodies [15]. Rheumatoid factors result in various modifications like citrullination (ACPA), carbamylation (aCarP), and acetylation (AAPA), as well as the immigration of T and B lymphocytes into the synovium [6]. The human leukocyte antigens genes are one of the strongest gene variants that predispose people to have RA [16]. By encouraging the production of inflammatory cytokines and chemokines, the innate immune system plays a significant role at the beginning of local inflammation, which ultimately contributes to the pathogenesis of RA [15]. Due to its high rate of morbidity and disability, RA places a significant cost on healthcare systems [17].

Scientists have conducted numerous clinical trials to investigate various medicines for the treatment of RA, some of which have been licensed for use in routine clinical practice. The medications that are widely used to treat RA include corticosteroids, disease-modifying anti-rheumatic drugs (DMARDs), and non-steroidal anti-inflammatory drugs [18]. In the present study, the correlation between tumor protein P53 (TP53) and interleukin-10 (IL10) with RA was examined. The tumor suppressor protein TP53 is essential for the cell's response to various stresses, such as DNA damage, hypoxia, nutrient deprivation, and oncogene activation. In addition, reactive oxygen species generation in cells is downregulated by p53, protecting against DNA damage and cell death brought on by oxidative stress [19]. The cytokine IL-10 is an essential facilitator of anti-inflammatory processes to protect a host against exaggerated reactions to infections and microbiota leading to the hypothesis that IL-10 might be a successful RA therapy. It also has important functions in several other situations, such as sterile wound healing, autoimmune disorders, cancer, and homeostasis [20]. The current study investigates the pharmacology study of NR against RA.

METHODS

Retrieval of ligands

The candidate ligands were chosen using the Drugbank (https://go.drugbank.com/) and Indian medicinal plants, phytochemistry, and therapeutics (https://cb.imsc.res.in/imppat/) databases. The Canonical simplified molecular-input line-entry system (SMILES) number of the top 24 ligands selected for the current experiment are downloaded in SDF format from PubChem (https://pubchem.ncbi.nlm.nih.gov/) database.

Retrieval of proteins

Using canonical NR smiles from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) database, the top 20 predicted target proteins of NR were chosen using the STITCH (http://stitch.embl.de/) database whereas known target proteins for RA were selected from the GeneCards (https://www.genecards.org/) database whose score is more than 40.

Construction of protein-protein interactions (PPI) network

The PPI construction is an important step of bioinformatics which help researchers to understand the level of interaction between two proteins [21]. The STRING (https://string-db.org/) platform was then used to import the probable targets mentioned above. Organism was restricted to "Homo Sapiens." Free nodes were hidden. The data with confidence levels higher than 0.90 were selected, the font size of the protein is set to 14 and disconnected nodes in the network were hidden. K-means clustering is used to cluster the network, with the number of clusters set to 3 and the edges between clusters maintained as dotted lines as default. Thus, PPI network was constructed to explore the interaction between NR-related targets and RA targets.

Gene ontology (GO) enrichment and pathway analysis

ShinyGO 0.76.3 (http://bioinformatics.sdstate.edu/go/) was used to conduct an analysis of GO and Kyoto Encyclopedia of Genes and Genomes pathway enrichment for clusters collected from the STRING database to obtain the possible pathways implicated. The best matching species is selected "Human". The significant terms and pathways were selected with the threshold of adjusted minimum p-value using FDR to 0.05.

Homology modeling

Homology modeling, in contrast to other approaches, is one of the most reliable and simple computer techniques for predicting the three-dimensional (3D) structure of a protein based on the amino acid composition of the protein [22]. The UniProt (https://www.uniprot.org/) database is used to retrieve the FASTA sequence of TP53 and IL10 proteins whose ID is K7PPA8 and P22301, respectively. SWISS-MODEL (https://swissmodel.expasy.org/) database is used to obtain the best model of TP53 and IL10 protein in protein data bank (PDB) format based on the GMEANDisCo and global mean quality estimate value. The template 2ilk.1.A IL10 with a sequence identity of 100% for IL10 and template 3q01.1.B Cellular tumor antigen p53 with a sequence identity of 92.70% for TP53 were selected to build the model. Ramachandran Plot and secondary structure of respective protein achieved from PDBsum Generate (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html) bio tool. ProSA-Web server (https://prosa.services.came.sbg.ac.at/prosa.php) is used to obtain the Z-Score graph of TP53 and IL10. The pure forms of the proteins TP53 and IL10, as well as a hydropathy plot and 3D protein structure, were obtained using the BIOVIA Discovery Studio Visualizer.

Protein purification

Both TP53 and IL10 proteins were purified by the BIOVIA Discovery Studio Visualizer software to reduce the complexity. Since water molecules can affect docking scores and obtain all possible binding sites, they were eliminated before docking. The pre-bound ligands are taken out of the crystal structures to speed up binding with the ligands selected for the investigation. B chains in TP53 and A chains in IL10 were left intact for analysis while additional chains were removed to simplify the protein structures. Due to the removal of all these results into destructions of charges, therefore polar hydrogen atoms were added to neutralize it and improve purified structures and download the purified protein in PDB format.

Molecular docking

For predicting interactions between the key active compound and the protein of the target, molecular docking is one of the most effective structure-based in-silico approaches [23]. The target proteins TP53 and IL10 were loaded into the PyRx tool as a macromolecule and NR derivatives were loaded as ligands. The PDB format of a protein is converted into PDBqt i.e., Kollmann charges are added and atoms are made as ADT4. The OPENBABEL was used to convert all the ligands. SDF format to. PDB file format. Using the Vina Wizard, the ligands were independently docked against TP53 and IL10. Energy minimization was carried out and converted all the ligands in PDBqt. The top 6 most effective compounds of respective protein-ligand interaction were chosen for visualization depending on their binding affinity value.

Visualization

To visualize the protein-ligand interaction, Using Dassault Systems BIOVIA Discovery Studio Visualizer, the conformations with the highest binding scores were downloaded.PDB format, and 3D models were produced.

Pharmacological studies

The top 14 ligands were chosen for pharmacological study based on molecular docking studies. SwissADME (http://www.swissadme.ch/) website is used to obtain the physiochemical qualities such as blood-brain barrier (BBB), GI absorption, permeability glycoprotein, synthetic accessibility, PAINS, H donors, H acceptors, Lipophilicity, size, polarity, saturation, and flexibility. The best ligands are selected using the LIPINSKI rule of five. To predict the toxicity of ligands ProTox-II (https://tox-new.charite.de/protox_II/) database is used.

RESULTS

TP53 protein structure analysis

The molecular function of proteins can be better understood by studying their 3-D structures, which have a wide range of applications in the field of life sciences [24]. PROCHECK was used to construct the alpha-synuclein Ramachandran plot which is shown in Figs. 1 and 2. The graph's red sections correspond to the sterically permitted zones that permit stable peptide structure. 201 amino acid residues, or 90.1%, fall in the most favored region, while 1 residue, or 0.4%, falls in the disallowed region. The additionally allowed regions and generously allowed regions account for 9.0% and 0.4%. Out of the 264 residues, 223 are non-proline and non-glycine residues, 18 are glycine residues, 21 are proline residues, and 2 are end residues. Protein TP53's predicted secondary structure includes 2 sheets, 3 beta hairpins, 3 beta bulges, 11 strands, 4 helices, 31 beta turns, and 3 gammas turn according to

the PDBsum data. The Z-Score value is −6.59 obtained from the ProSA web server (Fig. 1).

IL10 protein structure analysis

In the IL10 protein, there were 266 amino acid residues, or 93.0%, fell in the most favored region, while 1 residue, or 0.3%, fall in the disallowed region. The additionally allowed regions and generously allowed regions account for 4.2% and 2.4%. Out of the 310 residues, 286 are non-proline and non-glycine residues, 10 are glycine residues, 10 are proline residues, and 4 are end residues. Protein IL10 's predicted secondary structure includes 11 helices, 34-helix-helix interacts, 10 beta turns, 2 gamma turn, and 2 disulfides according to the PDBsum data. The Z-Score value is −3.53 obtained from the ProSA web server (Fig. 2).

PPI network

By Kmean default setting two major clusters was identified among which TP53 and IL10 are taken based on interaction done with neighboring protein which is carried out as a major target and subjected to the pathway analysis and molecular docking (Fig. 3).

Pathway analysis

Proteins in TP53 and IL10 clusters are subjected to pathway analysis to identify the top 5 pathways and these pathways for TP53 are thyroid cancer, bladder cancer, endometrial cancer, colorectal cancer, and adherens junction (Fig. 4). Similarly, pathways for IL10 are African trypanosomiasis, malaria, inflammatory bowel disease, viral protein interaction with cytokine and cytokine receptors, and pertussis (Fig. 5).

Drug likeliness analysis

A molecule must meet important criteria such as the Lipinski rule of 5, ADMET properties, and physicochemical characteristics before it can be used in the manufacturing of drugs. Prediction of toxicity is also essential for the creation of a drug with the fewest adverse effects.

Pharmacological studies

All the top ligands were investigated for their physicochemical properties including solubility, lipophilicity, flexibility and saturation (Table 1).

Lipinski filter analysis

The screening of drug molecules traditionally follows the Lipinski Filter analysis. From the results of present study (Table 2) it is evident that all majority of the Ligands fulfilled Lipinski filter analysis. Whereas, the ligands like Hesperidin and Naringenin 5-O-neohesperidoside had marginally higher molecular weight, But these compounds were considered due to their higher binding with the target proteins.

ADME analysis

There are four main components to an ADME analysis. The BBB limits the amount of the substance's intracranial movement BBB. To make a drug, you need to know this information. To maximize the drug's effectiveness, a significant quantity of GI adsorption is recommended. Furthermore, the substance must be readily soluble. Therefore, amounts with less negative solubility are accepted.

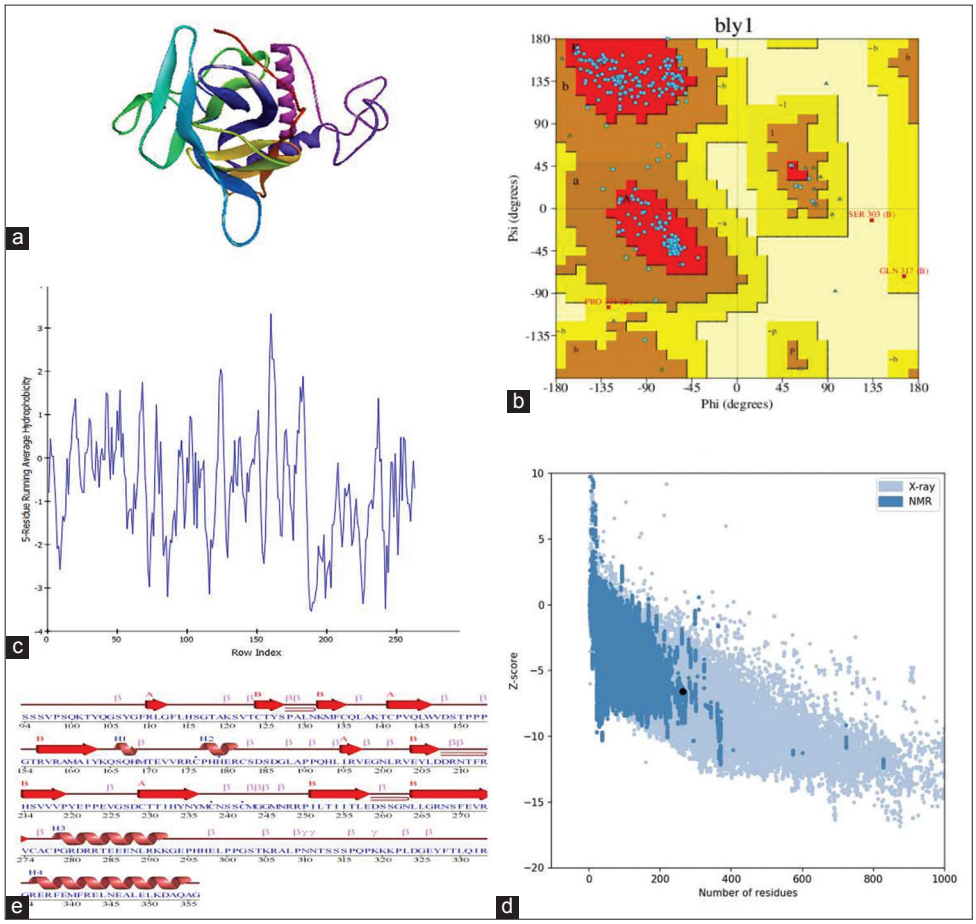

Fig. 1: TP53 protein structure analyzed by the following: (a) 3D structure of TP53 using BIOVIA Discovery Studio Visualizer. (b) Ramachandran plot using PDBsum generate. (c) Hydropathy plot using BIOVIA Discovery Studio Visualizer. (d) Z-Score graph using ProSA web server. (e) Secondary structure using PDBsum generate

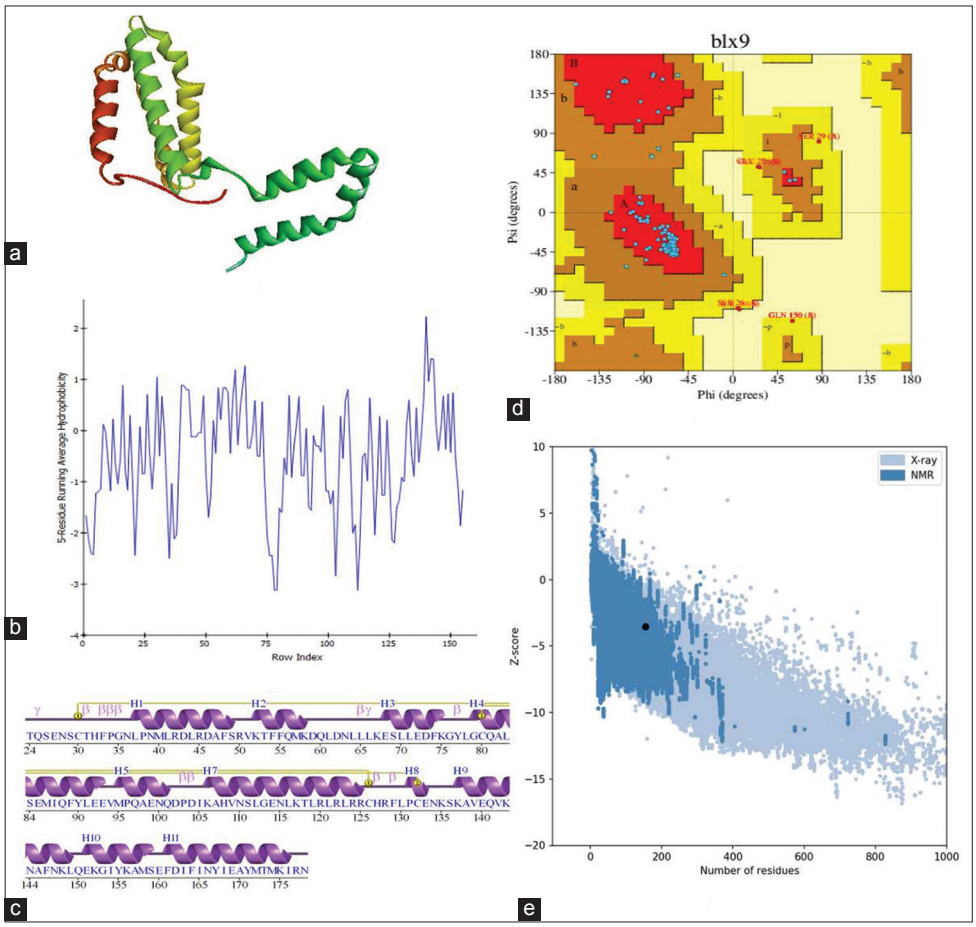

Fig. 2: IL10 protein structure analyzed by the following: (a) 3D structure of IL10 using BIOVIA Discovery Studio Visualizer. (b) Ramachandran plot using PDBsum generate. (c) Hydropathy plot using BIOVIA Discovery Studio Visualizer. (d) Z-Score graph using ProSA web server. (e) Secondary structure using PDBsum generate

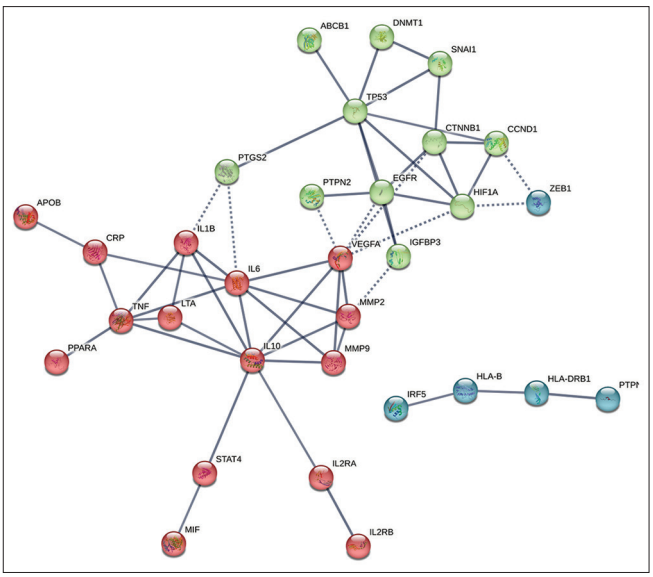

Fig 3. Protein-protein interaction between predicted targets and known targets using STRING webserver

Toxicity prediction

The following are some of the key characteristics of toxicity prediction. Hepatotoxicity, Carcinogenicity, Immunotoxicity, and Cytotoxicity.

Molecular docking

The binding affinity of all the selected ligands toward the TP53 and IL10 protein as obtained by PyRx is enlisted in Table 5.

Visualization

In the current analysis those ligands are docked whose binding affinity is better than -7 taken into consideration for visualization. From the Fig. 6a it is evident that ligand Hesperidin is making 11 interactions with amino acids of protein, that is, Lysine, Glutamine, Serine, 2 Asparagine, 2 Leucine, Glycine, Histidine, Tyrosine, and Proline. From Fig. 6b it is evident that ligand NR -7-rhamnoglucoside is making 6 interactions with amino acids of protein i.e., Glutamine, Serine, Threonine, Tyrosine, Proline, and Asparagine. From Fig. 6c it is evident that ligand Silibinin is making 6 interactions with amino acids of protein i.e., Lysine, 2 Asparagine, 2 Leucine, and Glutamic acid. From Fig. 6d it is evident that ligand Prunin is making 5 interactions with amino acids of protein, that is, Tyrosine, Threonine, 2 Asparagine, and Serine. From Fig. 6e it is evident that ligand Chalconaringenin 4'-glucoside is making 5 interactions with amino acids of protein, that is, Tyrosine, Asparagine, Phenylalanine, Serine, and Glutamine. From Fig. 6f it is evident that ligand Naringenin 4'-O-alpha-L-rhamnopyranoside is making 5 interactions with amino acids of protein, that is, 2 Asparagine, Serine, Leucine, and Glycine. It is found that Asparagine (Asn) is a common amino acid in the interaction of the top 6 ligands with TP53. It is also found that ligands interacting with the binding pocket atoms are Lysine, Leucine, Asparagine, and Glutamic acid, and this is the predominant interaction that can potentially inhibit the protein.

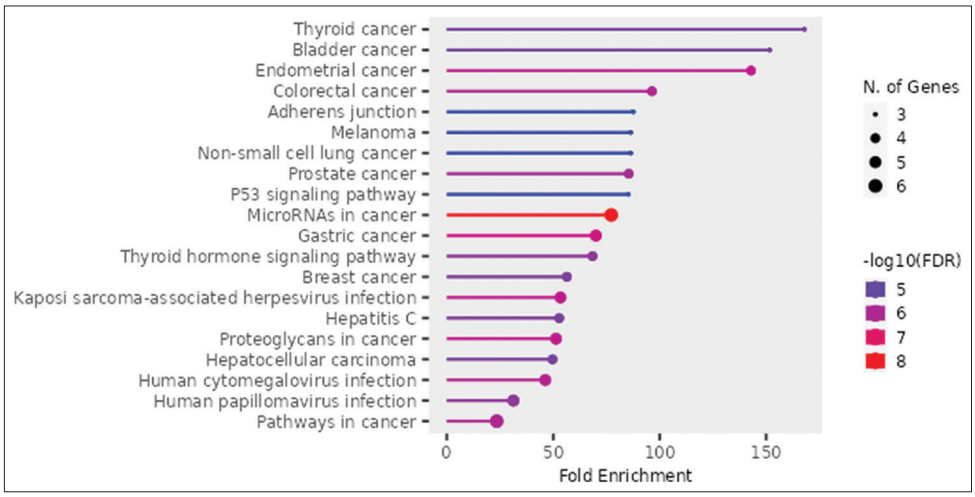

Fig. 4: Pathway analysis for the proteins mediated by TP53 cluster

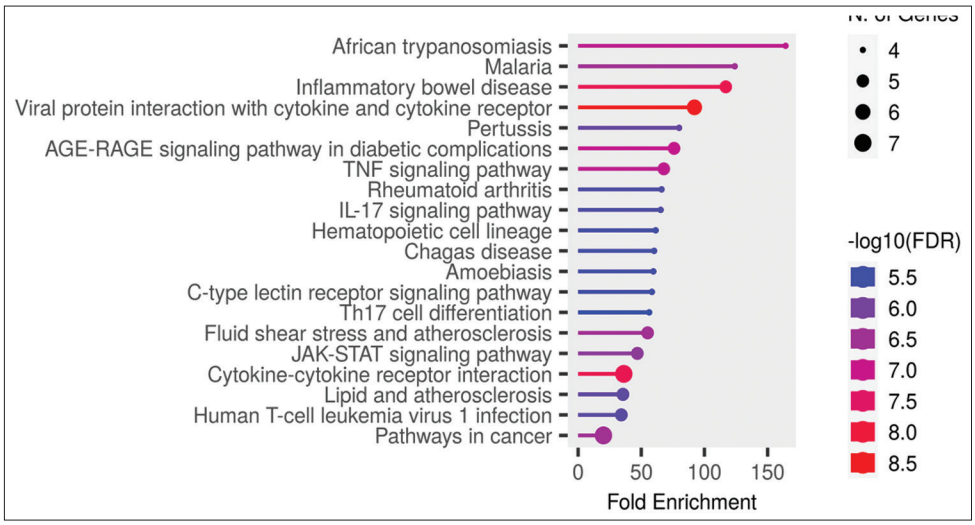

Fig. 5: Pathway analysis for the proteins mediated by IL10 cluster

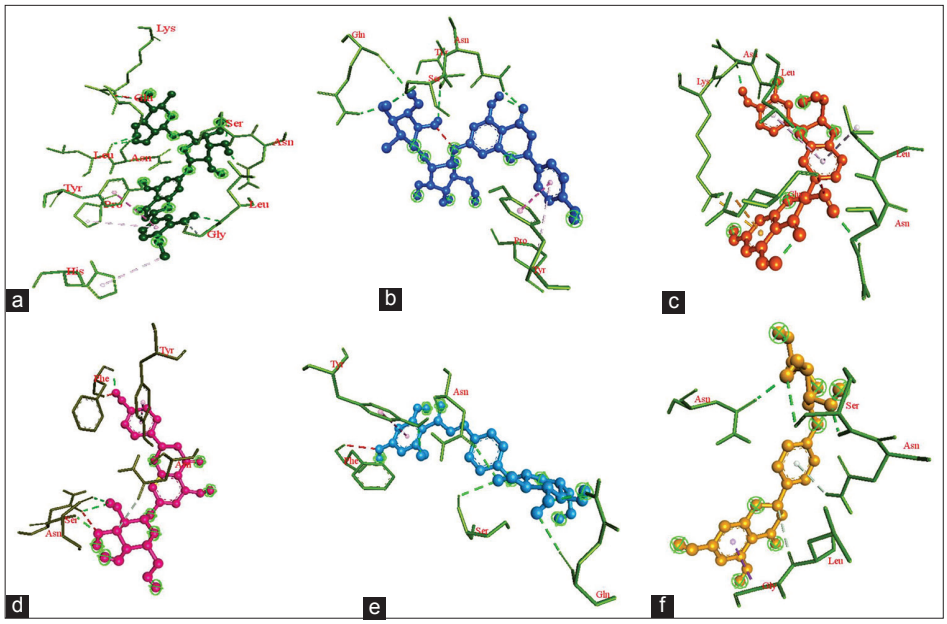

Fig. 6: Docking analysis of top 6 ligands with TP53 protein. Molecular docking of TP53 with (a) Hesperidin, (b) Naringenin-7-rhamnoglucoside, (c) Silibinin, (d) Prunin, (e) Chalconaringenin 4'-glucoside, and (f) Naringenin 4'-O-alpha-L-rhamnopyranoside, has demonstrated that have better docking affinity.

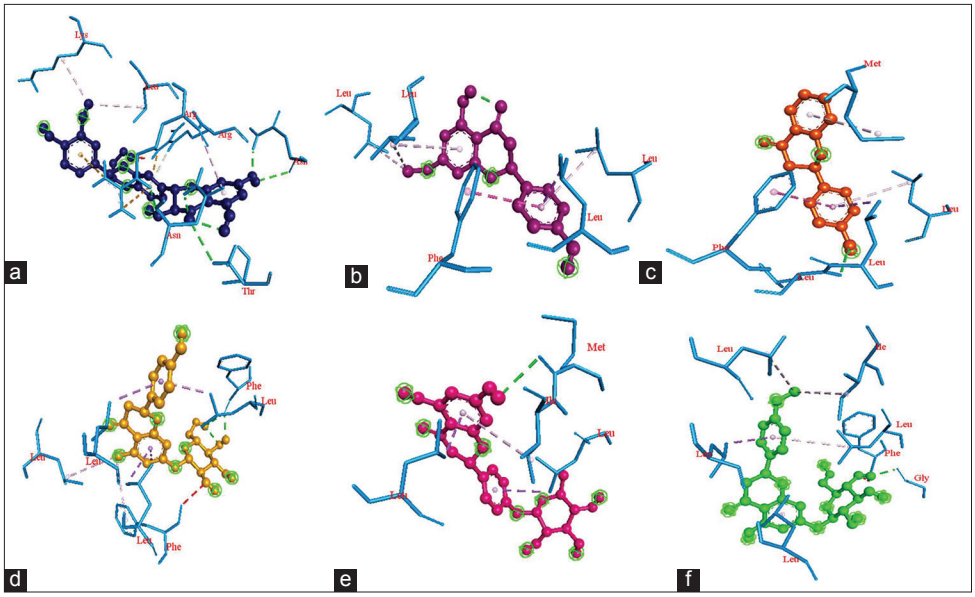

Fig. 7: Docking analysis of top 6 ligands with IL10 protein. Molecular docking of IL10 with (a) Silibinin, (b) Sakuranetin (c) 4'-Hydroxyflavanone, (d) Puddumin A (e) Naringenin 4'-O-alpha-L-rhamnopyranoside, and (f) Puddumin B, has demonstrated that have better docking affinity.

Table 1: Physicochemical properties

Ligand	MW	Fraction CSp3	Rotatable bond	TPSA	Lipophilicity
Naringenin-7-rhamnoglucoside	580.53	0.52	6	225.06	−0.44
Naringenin 4'-O-alpha-L-rhamnopyranoside	418.39	0.38	3	145.91	1.15
Silibinin	482.44	0.24	4	155.14	1.9
Puddumin-A	448.42	0.41	5	155.14	0.43
Puddumin B	448.42	0.41	5	155.14	0.98
Chalconaringenin 4'-glucoside	434.39	0.29	6	177.14	0.73
Hesperidin	610.56	0.54	7	234.29	−0.14
Prunin	434.39	0.38	4	166.14	0.65
Naringenin 5-O-neohesperidoside	580.53	0.52	6	225.06	−0.99
4'-Hydroxyflavanone	240.25	0.13	1	46.53	2.78
Sakuranetin	286.28	0.19	2	75.99	2.85
Naringenin 5-methyl ether	286.28	0.19	2	75.99	2.3
Naringetol	272.25	0.13	1	86.99	2.52
Naringenin 4'-O-glucoside	434.39	0.38	4	166.14	0.65

Table 2: Data for the properties of the Lipinski rule obtained using SwissADME

Ligand	Molecular weight	MLogP	H donors	H acceptors	Molar refractivity
Naringenin-7-rhamnoglucoside	580.53	−2.77	8	14	134.91
Naringenin 4'-O-alpha-L-rhamnopyranoside	418.39	−0.65	5	9	102.53
Silibinin	482.44	−0.4	5	10	120.55
Puddumin-A	448.42	−1.2	5	10	108.16
Puddumin B	448.42	−1.2	5	10	108.16
Chalconaringenin 4'-glucoside	434.39	−1.5	7	10	106.46
Hesperidin	610.56	−3.04	8	15	141.41
Prunin	434.39	−1.42	6	10	103.69
Naringenin 5-O-neohesperidoside	580.53	−2.77	8	14	134.91
4'-Hydroxyflavanone	240.25	1.85	1	3	67.52
Sakuranetin	286.28	0.96	2	5	76.04
Naringenin 5-methyl ether	286.28	0.96	2	5	76.04
Naringetol	272.25	0.71	3	5	71.57
Naringenin 4'-O-glucoside	434.39	−1.42	6	10	103.69

In the current analysis, those ligands are docked whose binding affinity is better than −6.5 taken into consideration for visualization. From Fig. 7a it is evident that ligand Silibinin is making 7 interactions with amino acids of protein, that is, Lysine, Leucine, 2 Arginine, Threonine, and 2 Asparagine. From Fig. 7b it is evident that ligand Sakuranetin is making 5 interactions with amino acids of protein, that is, 4 Leucine and Phenylalanine. From Fig. 7c, it is evident that ligand 4'-Hydroxyflavanone is making 5 interactions with amino acids of protein, that is, 3 Leucie, Phenylalanine. and Methionine. From Fig. 7d it is evident that ligand Puddumin A is making 6 interactions with amino acids of protein, that is, 4 Leucine and 2 Phenylalanine. From Fig. 7e it is evident that ligand Naringenin 4'-O-alpha-L-rhamnopyranoside is making 4 interactions with amino

Table 3: ADME data obtained using SwissADME

Ligands	BBB barrier	GI absorption	PGP substrate	Solubility (LOGSw-SILICOS IT)	Bioavailability	SA score
Naringenin-7-rhamnoglucoside	No	Low	Yes	−0.49	0.17	6.16
Naringenin 4'-O-alpha-L-rhamnopyranoside	No	Low	Yes	−2.28	0.55	4.92
Silibinin	No	Low	No	−4.5	0.55	4.92
Puddumin-A	No	Low	Yes	−2.39	0.55	5.1
Puddumin B	No	Low	Yes	−2.39	0.55	5.09
Chalconaringenin 4'-glucoside	No	Low	No	−0.94	0.55	4.9
Hesperidin	No	Low	Yes	−0.58	0.17	6.34
Prunin	No	Low	Yes	−1.71	0.55	4.98
Naringenin 5-O-neohesperidoside	No	Low	Yes	−0.49	0.17	6.24
4'-Hydroxyflavanone	Yes	High	Yes	−4.58	0.55	2.82
Sakuranetin	Yes	High	No	−4.12	0.55	3.11
Naringenin 5-methyl ether	Yes	High	Yes	−4.12	0.55	3.12
Naringetol	No	High	Yes	−3.42	0.55	3.01
Naringenin 4'-O-glucoside	No	Low	Yes	−1.71	0.55	4.96

BBB: Blood-brain barrier

Table 4: Toxicity data obtained using ProTox

Compound	Predicted LD 50 (mg/kg)	Predicted toxicity class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Cytotoxicity
Naringenin-7-rhamnoglucoside	2300	5	0.81	0.9	0.99	0.66
Naringenin 4'-O-alpha-L-rhamnopyranoside	2300	5	0.72	0.62	0.99	0.83
Silibinin	2000	4	0.78	0.72	0.97	0.77
Puddumin-A	2300	5	0.83	0.9	0.91	0.58
Puddumin B	2300	5	0.83	0.9	0.91	0.58
Chalconaringenin 4'-glucoside	4000	5	0.8	0.77	0.99	0.85
Hesperidin	12000	6	0.81	0.93	0.99	0.85
Prunin	2300	5	0.82	0.85	0.63	0.69
Naringenin 5-O-neohesperidoside	3000	5	0.81	0.9	0.98	0.66
4'-Hydroxyflavanone	2000	4	0.58	0.58	0.99	0.68
Sakuranetin	2000	4	0.71	0.52	0.51	0.71
Naringenin 5-methyl ether	2000	4	0.68	0.5	0.58	0.79
Naringetol	2000	4	0.67	0.62	0.88	0.59
Naringenin 4'-O-glucoside	2300	5	0.82	0.85	0.84	0.69

Table 5: Docking score of TP53 and IL10 protein with selected ligands

Protein	Ligand	Binding affinity
TP53	Hesperidin	−8.1
	Naringenin 4'-O-alpha-L-rhamnopyranoside	−7.6
	Silibinin	−7.5
	Naringenin-7-rhamnoglucoside	−7.2
	prunin	−7.2
	Chalconaringenin 4'-glucoside	−7.1
IL10	4'-Hydroxyflavanone	−7.1
	Naringenin 4'-O-alpha-L-rhamnopyranoside	−7.1
	Puddumin B	−7.1
	Silibinin	−7.0
	Sakuranetin	−7.0
	Puddumin A	−6.9

acids of protein i.e., 2 Leucine, Methionine, and Isoleucine. From Fig. 7f it is evident that ligand Puddumin B is making 7 interaction with amino acids of protein, that is, 4 Leucine, Phenylalanine, Glycine, and Isoleucine. It is found that Leucine (Leu) is a common amino acid in the interaction of the top 6 ligands with IL10. It is also found that ligands interacting with the binding pocket atoms are Lysine, Leucine, and Phenylalanine, and this is the predominant interaction that can potentially inhibit the protein.

DISCUSSION

The current therapy for RA and other immune-mediated diseases still lacks efficacy, and no recovery therapy has been found because treating arthritis involves multiple inflammatory factors, making medication challenges. As a result, there is still a high demand for robust biomarkers to objectively monitor inflammatory activity and response to therapy [15,25]. NR has been demonstrated to have therapeutic potential in studies using a variety of animal models for the treatment of several inflammation-related disorders, such as sepsis, fulminant hepatitis, fibrosis, and cancer [26]. Therefore, for the current study phytocompounds of NR were investigated to assess their potential as RA drug candidates. NR is mainly found in citrus fruits and tomatoes. Phenylalanine, an aromatic amino acid, can be used to create many conjugate forms of NR, and each of these forms has unique features relating to absorption, distribution, metabolism, and elimination. It has been noted that NR is efficiently absorbed by the intestinal tract, making it quickly accessible in the bloodstream [27].

In PPI network pathway analysis, we found that TP53 and IL10 are the major protein obtained from STRING database analysis. From the past research paper, we found that P53 controls several signaling pathways, including apoptosis, cell cycle, DNA repair, cellular stress responses, induces the production of cytokines, promotes the expression of matrix metalloproteinase, and expression of Toll-like receptors as well as the maturation and differentiation of innate and adaptive immune cells. P53 controls several anti- and pro-apoptotic genes to modulate the apoptosis and proliferation processes. Due to p53's crucial involvement in cell proliferation, apoptosis, and inflammation, its dysfunction plays a significant part in the pathophysiology of RA [28]. Pro-inflammatory cytokines including TNF, IL-1, IL-6, and IL-10, which are generated by inflamed synovial regions in RA, are known to affect osteoclast and osteoblast development and affect bone. However, the protective impact of IL-10 seems to be reduced in RA. It has been discovered that

high levels of IL-10 play a paradoxical function in the development of RA in patients [29]. Further TP53 and IL-10 were subjected to pharmacological studies for drug design.

In the current investigation, 14 phytocompounds from NR were used, and they were chosen because of their high binding energy. All 14 compounds underwent stringent pharmacological analysis and were assessed for various parameters like physicochemical properties, sp3 hybridization, rotatable bond, toxicity, no of hydrogen atom donors and acceptors, molecular weight, etc. From the results of these analyses, it is evident that all the selected compounds had druggable parameters and passed the filters of Lipinski and ADMET analysis. However, these compounds displayed varied GI absorption and BBB permeability. The current study hypothesized that only those substances - 4′-Hydroxyflavanone, Sakuranetin, and NR 5-methyl ether with high gastrointestinal absorption and blood barrier permeability would be taken into consideration as an oral medication to treat RA. It is also found that these ligands found to be nontoxic fall under class 4 from the ProTox database which means that these drugs are not fatal. However, it was revealed that saturation, which depends on the sp3 hybridization of the carbon atom of these three ligands, is <0.25. Among these three, 4′-Hydroxyflavanone and Sakuranetin ligands had a high binding affinity and also had better interaction with protein complexes as they interact with important amino acids. According to earlier studies, Sakuranetin has anti-inflammatory and antioxidant properties. Sakuranetin's anti-inflammatory properties may be influenced by NF-B activation, which is the principal regulator of pro-nociceptive factors and controls the transcription of numerous genes, including cytokines and iNOS. The anti-inflammatory properties of sakuranetin may be related to the presence of a hydroxyl group at C-7 and a methoxyl group at C-5 in this molecule [30]. Therefore, it can be concluded that the ligands 4′-Hydroxyflavanone and Sakuranetin can be developed as a drug against RA disease.

CONCLUSION

RA is an autoimmune inflammatory disease that not only damages joints but also other body organs and produces excruciating pain. Severe RA can still result in physical limitations, although new pharmaceutical types have significantly improved treatment possibilities. Numerous medications are being developed to reduce the symptoms of RA. Medicinal plants are one of the types that can be used to make medications to treat RA. One such plant with numerous Pharmacological properties is NR and its derivatives. In this approach, improved prevention and treatment can be achieved with the help of several studies. Because of the growing use of medicinal plants in medication development, medicine will undergo a substantial change.

ACKNOWLEDGMENT

I hereby acknowledge Ms. Susha Dinesh for her guidance and BioNome for providing computational facilities and support in the scientific research services.

REFERENCES

1. Kabra A, Garg R, Brimson J, Živković J, Almawash S, Ayaz M, *et al*. Mechanistic insights into the role of plant polyphenols and their nano-formulations in the management of depression. Front Pharmacol 2022;13:1046599.
2. Duda-Madej A, Stecko J, Sobieraj J, Szymańska N, Kozłowska J. Naringenin and its derivatives-health-promoting phytobiotic against resistant bacteria and fungi in humans. Antibiotics 2022;11:1628.
3. Salehi B, Fokou PV, Sharifi-Rad M, Zucca P, Pezzani R, Martins N, *et al*. The therapeutic potential of naringenin: A review of clinical trials. Pharmaceuticals (Basel) 2019;12:11.
4. Ionescu CE, Popescu CC, Agache M, Dinache G, Codreanu C. Depression in Rheumatoid arthritis: A narrative review-diagnostic challenges, pathogenic mechanisms and effects. Medicina (Kaunas) 2022;58:1637.
5. Smesam HN, Qazmooz HA, Khayoon SQ, Almulla AF, Al-Hakeim HK, Maes M. Pathway phenotypes underpinning depression, anxiety, and chronic fatigue symptoms due to acute rheumatoid arthritis: A precision nomothetic psychiatry analysis. J Pers Med 2022;12:476.
6. Scherer HU, Häupl T, Burmester GR. The etiology of rheumatoid arthritis. J Autoimmun 2020;110:102400.
7. Slagter L, Demyttenaere K, Verschueren P, De Cock D. The effect of meditation, mindfulness, and yoga in patients with rheumatoid arthritis. J Pers Med 2022;12:1905.
8. Metsios GS, Kitas GD. Physical activity, exercise and rheumatoid arthritis: Effectiveness, mechanisms and implementation. Best Pract Res Clin Rheumatol 2018;32:669-82.
9. Aletaha D, Smolen JS. Diagnosis and management of rheumatoid arthritis: A review. JAMA 2018;320:1360-72.
10. Prasad P, Verma S, Surbhi, Ganguly NK, Chaturvedi V, Mittal SA. Rheumatoid arthritis: Advances in treatment strategies. Mol Cell Biochem 2022;478:1-20.
11. Ma C, Wang J, Hong F, Yang S. Mitochondrial dysfunction in rheumatoid arthritis. Biomolecules 2022;12:1216.
12. Radu AF, Bungau SG. Management of rheumatoid arthritis: An overview. Cells 2021;10:2857.
13. Tanase DM, Gosav EM, Petrov D, Teodorescu DS, Buliga-Finis ON, Ouatu A, *et al*. MicroRNAs (miRNAs) in cardiovascular complications of rheumatoid arthritis (RA): What is new? Int J Mol Sci 2022;23:5254.
14. Yang G, Kang HC, Cho YY, Lee HS, Lee JY. Inflammasomes and their roles in arthritic disease pathogenesis. Front Mol Biosci 2022;9:1027917.
15. Inciarte-Mundo J, Frade-Sosa B, Sanmartí R. From bench to bedside: Calprotectin (S100A8/S100A9) as a biomarker in rheumatoid arthritis. Front Immunol 2022;13:1001025.
16. Mueller AL, Payandeh Z, Mohammadkhani N, Mubarak SM, Zakeri A, Bahrami AA, *et al*. Recent advances in understanding the pathogenesis of rheumatoid arthritis: New treatment strategies. Cells 2021;10:3017.
17. Zhu Y, Zhao T, Liu M, Wang S, Liu S, Yang Y, *et al*. Rheumatoid arthritis microenvironment insights into treatment effect of nanomaterials. Nano Today 2022;42:101358.
18. Huang J, Fu X, Chen X, Li Z, Huang Y, Liang C. Promising therapeutic targets for treatment of rheumatoid arthritis. Front Immunol 2021;12:686155.
19. Kang R, Kroemer G, Tang D. The tumor suppressor protein p53 and the ferroptosis network. Free Radic Biol Med 2019;133:162-8.
20. Saraiva M, Vieira P, O'Garra A. Biology and therapeutic potential of interleukin-10. J Exp Med 2020;217:e20190418.
21. Tang R. Improved dynamic PPI network construction and application of data mining in computer artificial intelligence systems. Sci Programming 2022;2022:2729401.
22. Muhammed MT, Aki-Yalcin E. Homology modeling in drug discovery: Overview, current applications, and future perspectives. Chem Biol Drug Des 2019;93:12-20.
23. Pinzi L, Rastelli G. Molecular docking: Shifting paradigms in drug discovery. Int J Mol Sci 2019;20:4331.
24. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, *et al*. SWISS-MODEL: Homology modelling of protein structures and complexes. Nucleic Acids Res 2018;46:W296-303.
25. Lim YY, Zaidi AM, Miskon A. Composing on-program triggers and on-demand stimuli into biosensor drug carriers in drug delivery systems for programmable arthritis therapy. Pharmaceuticals (Basel) 2022;15:1330.
26. Zeng W, Jin L, Zhang F, Zhang C, Liang W. Naringenin as a potential immunomodulator in therapeutics. Pharmacol Res 2018;135:122-6.
27. Arafah A, Rehman MU, Mir TM, Wali AF, Ali R, Qamar W, *et al*. Multi-therapeutic potential of naringenin (4',5,7-Trihydroxyflavonone): Experimental evidence and mechanisms. Plants (Basel) 2020;9:1784.
28. Taghadosi M, Adib M, Jamshidi A, Mahmoudi M, Farhadi E. The p53 status in rheumatoid arthritis with focus on fibroblast-like synoviocytes. Immunol Res 2021;69:225-38.
29. Hernández-Bello J, Oregón-Romero E, Vázquez-Villamar M, García-Arellano S, Valle Y, Padilla-Gutiérrez JR, *et al*. Aberrant expression of interleukin-10 in rheumatoid arthritis: Relationship with IL10 haplotypes and autoantibodies. Cytokine 2017;95:88-96.
30. Cruz MP, Andrade CM, Silva KO, de Souza EP, Yatsuda R, Marques LM, *et al*. Antinoceptive and anti-inflammatory activities of the ethanolic extract, fractions and flavones isolated from *Mimosa tenuiflora* (Willd.) Poir (Leguminosae). PLoS One 2016;11:e0150839