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Research Article

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In silico docking analysis of Janus kinase enzymes and phytochemicals

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ABSTRACT

Emerging challenges on outspread of diseases are accelerating extensive research on the discovery of new drugs. For this purpose, phytochemicals with its long history of traditional use are now being utilized as potential sources due its efficacy and safety. The application of computational biology is further aided to improve the process of initial screening through its fast, convenient and cost effective approach. Janus kinase is a key enzyme in the JAK - STAT pathway leading to various inflammatory diseases. In this study in silico enzyme-inhibitor binding simulation experiment was performed between eight phytochemicals and Janus kinase enzymes (JAK 1, 2 and 3) using the Patchdock docking server. Curcumin was found to have strongest binding potential when compared with other test chemicals. Random rotamers of curcumin improved its specificity towards specific JAK. The results suggested that in future this compound can be utilized therapeutically as a natural occurring JAK inhibitor.

Keywords: phytochemicals, *in silico*, docking, Janus kinase, curcumin

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INTRODUCTION

Janus kinase enzymes (JAK1, JAK2, and JAK3) play pivotal roles in transmitting various cytokines mediated signals via JAK-STAT pathway, such as erthyopoietin, IL6, IL2, prolactin and growth promotion factors (granulocyte-macrophage colony stimulating factor) [1]. Despite having an essential physiologic role, cytokine activities via JAK-STAT pathway involve in several patho-physiological disorders like autoimmune diseases, rheumatoid arthritis, psoriasis, myeloproliferative syndromes and cardiovascular disease [2]. Inhibition of JAK is thus a suitable therapeutic option for treatment of such diseases. JAK inhibitors are attracting a great deal of scientific attentions for developing new targeted therapies. However, toxicity associated with the synthetic inhibitors is emerging as potential challenges for designing small molecule JAK specific inhibitors [3]. The present work is motivated by the fact that there is a constant urge for developing new JAK inhibitors with more efficacy and less side effects than the currently available drugs.

Earlier scientific investigations have indicated the essential role of natural products of plant origin as potential sources of various advance pharmaceutical formulations [4]. Phytochemicals constitute a large group of chemical classes which include flavonoids, terpenoids and curcuminoids. These compounds have been reported to exert various biological functions such as antioxidant, anti genotoxic, anti cancer, anti diabetic, anti allergic, antimicrobial, antiviral and anti inflammatory properties [5-7]. Current research on phytochemicals is being targeted towards the discovery of new chemical entities to be used as drug leads.

The purpose of this study is to identify a novel JAK inhibitor using fast and convenient *in silico* molecular docking approach. Eight (8) phytocompounds selected from Human metabolome database with drug like properties, namely curcumin, beta farnesene, terpinen-4-ol, beta caryophyllene, curzerene, zingiberene, kaempferol and zingiberene had been screened for possible inhibition potential against three types of Janus kinase enzymes (JAK1, JAK2 and JAK3). Computational investigation of this study indicated curcumin as a potent inhibitor of JAK.

EXPERIMENTAL SECTION

Target proteins

The three dimensional structure of the enzymes JAK1, JAK2 and JAK3 deposited as x-ray diffraction data with the resolution of 2.73, 2.20 and 2.30 Å respectively were downloaded from protein data bank (PDB; http://www.rcsb.org/pdb/home/home.do) . The ligands from the proteins were removed and the structures were refined by the software UCSF-chimera (**Figure 1)**.

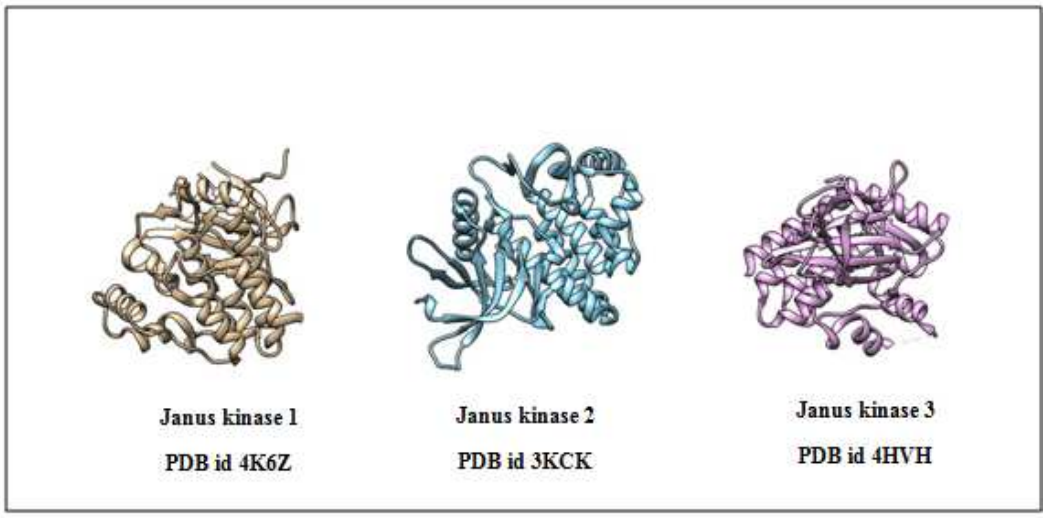

Figure 1 Three dimensional (3D) ribbon structures of the proteins used for docking study

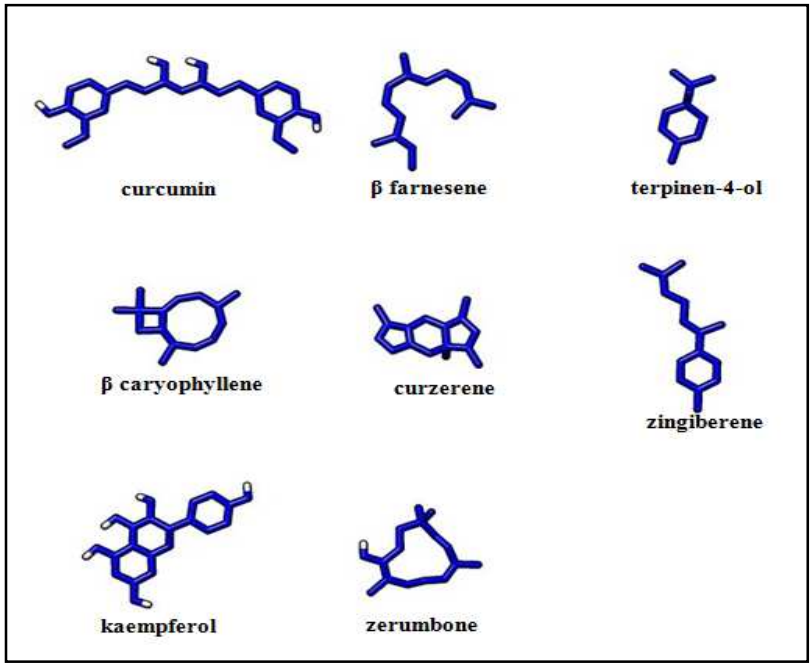

Figure 2 3D structures of phyto-compounds used for docking study

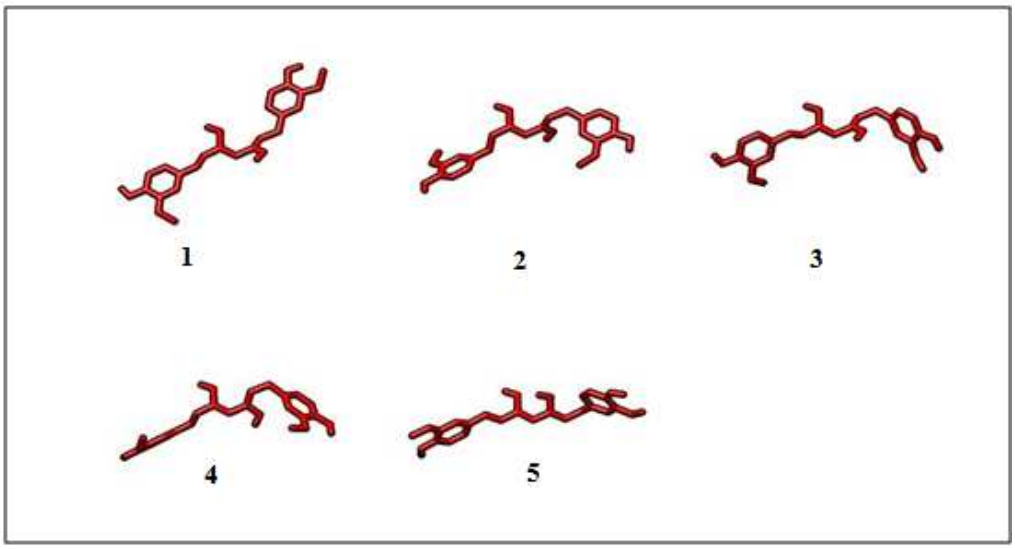

Figure 3 3D structures of curcumin rotamers

Ligands

Eight compounds of botanical origin namely curcumin, beta farnesene, terpinen-4-ol, beta caryophyllene, curzerene, zingiberene, kaempferol and zingiberene were selected for this study. The three dimensional (3D) PDB structures of the ligands were downloaded from Human metabolome database (HMDB; http://www.hmdb.ca) [8] (**Figure 2**). The structures were protonated using Avogadro software. Further five random rotamer conformers of curcumin were created using Avogdro software [9] (**Figure 3)**.

Determination of binding sites

Experimental ligand binding sites of the JAKs from PDB raw files were used as the predicted protein binding sites for the phyto-chemical ligands. These sites were determined by analyzing the raw PDB JAK-inhibitor complexes using LPC CSU server (http://ligin.weizmann.ac.il/lpccsu).

Drug evaluation and drug likeness

Human metabolome database (HMDB) is a database which provides detail information about the small molecule metabolites found in the human body. All the ligand molecules downloaded from HMDB were searched for metabolic information. Further, Molinspiration tool (http://www.molinspiration.com) was used to assess the drug like properties of the ligands. Drug like activities of the compounds were determined using Lipinski's rule of 5. The rules evaluate the solubility, permeability and bioavailability of the compounds. The Lipinski's rules of 5 stated that those ligands whose molecular weight is less than 500 g/moL, H bond donors less than 5, H bond acceptors less than 10 and log p-value less than 5 are considered to have drug like properties.

Computation of docking scores between the inhibitors (ligands) and JAKs

Patchdock server (http://bioinfo3d.cs.tau.ac.il/PatchDock) was used to compute the scores of the docked complexes. The server relies on the principle based on on molecular shape representation, surface patch matching plus filtering and scoring [10-11]. 3D structures of all three individual Janus kinases (JAK1, JAK2 and JAK3) and phytochemical inhibitors including curcumin rotamers were submitted in PDB format with enzyme-inhibitor parameter in Patchdock. Receptor binding site for individual protein was submitted as an additional input. Individual synthetic ligands and UCSF-Chimera refined enzymes (JAK1, JAK2 and JAK3) were re-docked and the scores obtained were served as controls for other docking experiments.

RESULTS AND DISCUSSION

Natural compounds of botanical origin are recently implemented as therapeutic substances over the conventional synthetic ones due its efficacy and safety. Covering a broad range of biological activities these compounds are successfully employed to treat numerous patho- physiological indications. Phyto-compounds categorized into various chemical classes such as flavonoids, terpenoids and curcuminoids are predominantly used as antioxidant

agents and widely applied to mitigate diseases in relevance with oxidative stress [12-13]. However, other pharmacological properties of phytochemicals have also been exploited by several scientific investigations. Anti-inflammatory properties of phytochemicals might be cited as examples [14-15].

Inflammation is a required immune responsive phenomenon, considered deleterious when causes physiological imbalance leading to the onset of an array of diseases such as rheumatoid arthritis, psoriasis, myeloproliferative syndromes and cardiovascular disease [2]. Janus kinases are the pro inflammatory enzymes that mediate the immune responses of multiple cytokines as well as various growth factors through the JAK-STAT signaling pathway [16]. The binding of an inflammatory signal to its receptor induces the phosphorylation of receptor associated JAKs (JAK1/JAK2/JAK3), which in turn phosphorylates STAT. The activated STAT forming homo or hetero dimmers, translocate to the nucleus where they directly bind to the promoter region of the specific inflammatory associated gene and regulate its entire transcription process. Inhibition of the activity of JAK can thus disable the chain of events in the respective inflammatory signal pathway [17-18].

Ruxolitinib, a small molecule JAK (JAK1 and JAK2) inhibitor, is frequently prescribed to treat patients with myelofibroses to reduce inflammatory symptoms. A long term clinical study using this drug in myelofibroses patients revealed its adverse side effects which include thrombocytopenia, worsening of anemia and serious withdrawal symptoms [3]. Considering the toxic effects of currently used JAK inhibitors eight compounds of botanical origin, namely curcumin, β farnesene, terpinen-4-ol, β caryophyllene, curzerene, zingiberene, kaempferol and zerumbone were chosen to analyze their inhibitory potentials of JAK enzymes (JAK 1, JAK 2 and JAK 3) for the development of safe and effective anti-inflammatory drugs using *in silico* approach.

Table 1 Binding site analysis

Enzymes	Synthetic inhibitors	Binding sites
JAK1 (PDB id 4K6Z)	8-oxo-pyridopyrimidine (PDB id 1Q3)	879ARG, 881LEU, 882GLY, 883GLU, 884GLY, 889VAL, 906ALA, 938VAL, 956MET, 957GLU, 958PHE, 959LEU,962GLY,963SER, 966GLU,1007ARG,1008ASN,1010LEU,1020GLY,1021ASP
JAK2 (PDB id 3KCK)	3,4-ring fused 7-azaindole (PDB id 3KC)	855LEU,856GLY,863VAL,880ALA,882LYS,898GLU,902LEU,911VAL,927LEU,929MET,930GLU, 931TYR,932LEU,935GLY, 936SER,980ARG,983LEU,993GLY,994ASP,995PHE
JAK3 (PDB id 4HVH)	Cyclo propyl pyrrolopyrazine (PDB id 19R)	828LEU,829GLY,830LYS,836VAL,853ALA,855LYS,884VAL, 902MET, 903GLU, 904TYR, 905LEU, 906PRO,908GLY,909CYS, 953ARG,954ASN,956LEU,966ALA,967ASP

Curcumin is prevalent in the species of the genus *Curcuma*, a Zingiberaceae member [19], whereas, zingiberene and kaempferol along with zerumbone were reported as major constituents in the rhizomes of *Zingiber officinale* [20] and *Z. zerumbet* [21-22] respectively. Others compounds are commonly found in the essential oils of different aromatic plants, however dominant in the rhizomes of Zingiberaceae species [23]. These phyto-compounds are noted to possess various medicinal properties including anti inflammatory applications in the ayurvedic practices as well as in the modern scientific explorations [24].

The efficacy of the bioactive compounds is often depreciated due to erratic cellular absorption resulting in poor bioavailability [25]. The size, chemical structures and the solubility of the compounds are known to physically influence such availability to the cells [26]. Therefore, evaluation of properties involving molecular chemistry is essential to consider a chemical entity to be used as a drug. In the present study of eight phyto-compounds with the exception of log P values, all met the drug like properties (**Table 2)**.

Computational docking analysis to study enzyme-inhibitor complexes has become a powerful tool in new drug discovery programs and is also a method for rapid identification of enzyme inhibitors with high precision. Phenolic compounds, widely abundant in herbs and spices have been previously exhibited to inhibit various enzymes using *in silico* docking approach. For an example, anthocyanins from blueberry-blackberry wine blends could bind to the active site of a diabatic enzyme Dipeptidyl Peptidase-IV [27]. In the present study, rigid body docking analysis mode was followed using Patchdock server. High docking score of the enzyme-inhibitor complex represents high binding capacity of the inhibitor to the enzyme. The procedure of such docking analysis was successfully employed by Purohit et al., 2008 [28] to study the binding affinity of Asp25 of HIV-1 protease mutants.

Table 2 Molecular properties of phytochemicals

No.	Compounds	HMDB* id	Cellular localization	Biofluid location	MW (g/moL)	logP	H bond donor	H bond acceptor
1	Curcumin	HMDB 02269	M **	Blood	368.38	2.3	2	6
2	β Farnesene	HMDB 35913	EC, M[#]	-	204.35	5.84	0	0
3	Terpinen-4-ol	HMDB 35833	EC, M	-	154.25	2.6	1	1
4	β Caryophyllene	HMDB 36792	EC, M	-	204.35	5.17	0	0
5	Curzerene	HMDB 38147	M	-	216.32	4.54	0	1
6	Zingiberene	HMDB 36164	EC, M	-	204.19	5.12	0	0
7	Kaempferol	HMDB 05801	M	Blood, urine	286.24	2.17	4	6
8	Zerumbone	HMDB 36667	EC, M	-	218.17	4.2	0	1

***HMBD:** Human metabolome database; [#]**EC:** extracellular,**M:** membrane*

The results of docking analysis suggested that the curcumin had a strong binding affinity towards all the three Janus kinases (JAK 1, JAK2 and JAK3) (**Table 3)**. The efficacy of curcumin to inhibit JAK 1, 2 and 3 at a rate comparable to the positive control ligands indicated that this phenolic compound might act as a natural inhibitor of Janus kinase enzymes. Among the other compounds tested, β farnesene, curzerene, kaempferol and zerumbone scored reasonably when compared to the respective controls. It is interesting that terpinen-4-ol and zingiberene scored low in docking analysis for all three types of JAKs. One explanation is that terpinen-4-ol with its small size and zingiberene without having either of H donor or acceptor bonds were unable to fit into the active site pockets of JAKs and lacked any structural interactions with the enzymes through H bonds respectively.

Table 3 Docking scores

Compounds	JAK1	JAK2	JAK3
Control ligand	4788	4768	4758
Curcumin	**5052**	**4912**	**4882**
β Farnesene	4262	4142	3860
Terpinen-4-ol	3576	3334	3288
β Caryophyllene	4216	4090	3950
Curzerene	4234	4002	3942
Zingiberene	3914	3908	3854
Kaempferol	4270	4226	4160
Zerumbone	4248	4132	4054

Table 4 Docking scores of Janus kinase (JAK)-curcumin rotamers

Compounds	JAK1	JAK2	JAK3
Curcumin rotamer 1	5476	5610	4890
Curcumin rotamer 2	5292	**5478**	**5194**
Curcumin rotamer 3	**5844**	5366	4842
Curcumin rotamer 4	5504	5460	4674
Curcumin rotamer 5	5334	5070	4658

Curcumin is a phenolic compound and a major component of turmeric (*C. longa*) and mango ginger (*C. amada*) [19]. The rhizomes of these plants are popularly used as spices in South Asian cuisines and also extensively used in the traditional medicines. This compound has recently received much attention due to its pharmacological properties such as anti-oxidant, anti-genotoxic and anti-inflammatory properties [29-30]. The anti-inflammatory mechanism of curcumin was attributed to the suppression of pro-inflammatory cytokines and their mediators such as TNF-γ, IL-1β and NO [18]. A study by Kim et al., 2003 [18] showed that curcumin could inhibit the catalytic role of Janus kinase by its association with the SH2 domain of JAK. In consistent with this finding, our data revealed that curcumin possessed the best binding potential to the JAKs above other seven phytochemicals screened for this experiment. However, specificity and selectivity of the inhibitor curcumin to a particular JAK remained a question in our study. To address this question, five random structural rotamers of curcumin were formed and subsequently docked with

JAKs (**Table 4, Figure 4)**. The docking calculation revealed that refinement of flexible bonds might offer specificity of an inhibitor to a particular enzyme, although extensive research is warranted.

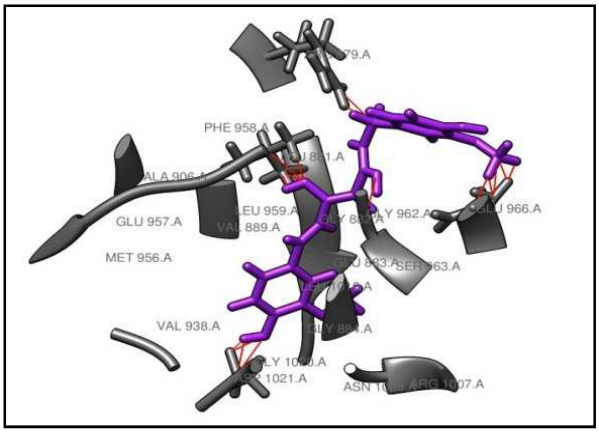

Figure 4A Rotamer 3-JAK1

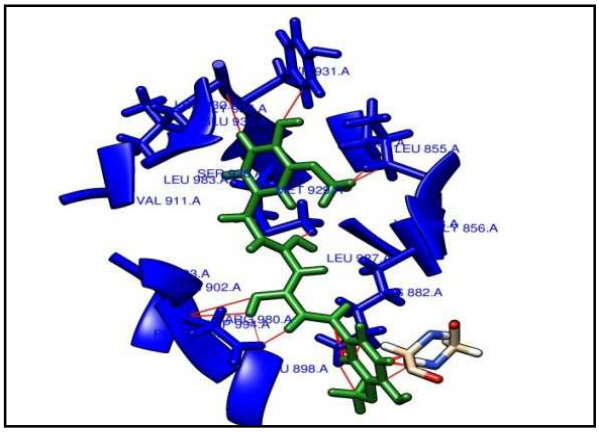

Figure 4B Rotamer 2-JAK2

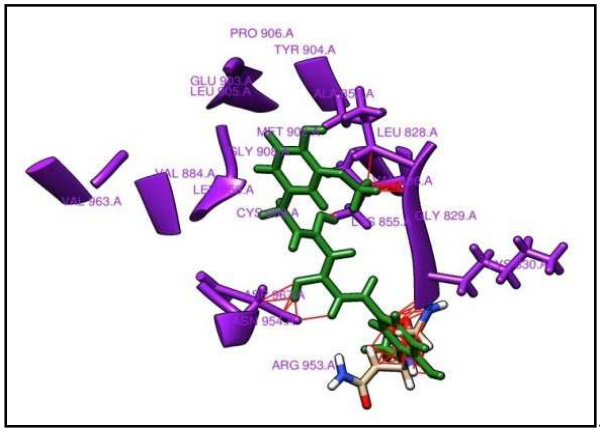

Figure 4C Rotamer 2-JAK3

Figure 4 Intermolecular interactions of Janus kinase 1 and curcumin rotamers in 3D space

CONCLUSION

Our study demonstrated that curcumin could bind strongly with JAK enzymes. Nevertheless, variation in structural conformations might give specificity to curcumin for binding with specific JAK. In general it can be concluded that the information obtained from this study can be further used for therapeutic purposes.

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