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MAPK signaling protein molecular target of *Cordia macleodii* Phytochemicals for Prevention of Chronic Diseases

Mohammad Nadeem Khan
Sos in Biotechnology, Bastar university, Dharapura, Jagdalpur, Chattisgarh (India) 494001

Abstract: The mitogen-activated protein kinases (MAPKs) pathways are activated by diverse extracellular and intracellular stimuli including peptide growth factors, cytokines, hormones, and various cellular stressors such as oxidative stress and endoplasmic reticulum stress .It is extensively verified that continued oxidative stress and oxidative damage may lead to chronic inflammation, which in turn can mediate most chronic diseases including cancer, Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) ,diabetes, cardiovascular, neurological, inflammatory bowel disease and pulmonary diseases. Current study was totally based on the screening of phytochemicals to find out the MAPKs receptor having strong binding affinity. Present study exhibited that two molecules coded cordia-1(4 5)-1formy 1-4-dihydroxy-3oxo 3-4 dihydro-2H-pyran-1-ium) and cordia-2 (5,7-dihydroxy-2{3-hydroxy-5methyl phenyl] 2-3 dihydroxy -4H benzopyran) kushenol K, silybin, taxifolin 3-O) have successful and potential binding with the target molecule. From the results showed the interactions between MAPKs protein receptor with phytocompounds, cordia-1 showed the best glide docking score -7.9 kcal/mol and the RMSD lower 42.01 ,upper value of -68.81 kcal/ . Based on the result, the cordia-1 target were run on MD simulations stable at 10 ns. Finally, this study concludes the cordia-1 is a suitable drug candidate for MAPKs blocked pathway and reduction the super radical generation.

Key words: - ligands, molecular docking, active site, binding affinity, pathological disorder.

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I. INTRODUCTION

In India, medicines based on plant origin have been the basis of treatment and cure for various pathogenic disorders [1]. A large number of plants are used by folklore traditions in India for treatment of various chronic diseases like s heart disease, stroke, cancer, chronic respiratory diseases and diabetes. [1]. Recently, the traditional use of plants for chronic disorder has received much attention from the scientific community. Herbal drugs are prescribed widely because of their effectiveness, low side effects and relatively low cost [2]. Therefore investigation on active principles from traditional medicinal plants has become more important [3].The world health organization [4] has also recommended the evaluation of the effectiveness of plants in treatments where we lack safe modern drugs [5].

Cordia macleodii Hook. (Boraginaceae), native to India, is a small sized tree. It has been reported that the tribal people use this plant as an aphrodisiac and also to treat mouth sores and jaundice[1]. Leaf of this plant is being reported as a wonderful wound healing drug[2]. Researchers have been carried out to evaluate hepatoprotective activity[3], pharmacognostical evaluation[4] and pharmacological evaluation of wound healing activity[5] of its leaf. Though the stem bark of this plant has been highlighted for different enthno-pharmacological properties [6]

a variety of drug discovery programs, to study complex biological and chemical systems. The integration of computational and experimental strategies has been of great value in the identification and development of novel promising compounds[7]. Broadly used in modern drug design, molecular docking methods explore the ligand conformations adopted within the binding sites of macromolecular targets. This approach also estimates the ligand-receptor binding free energy by evaluating critical phenomena involved in the intermolecular recognition process. . Therefore, a breakthrough in chronic disorder drug development is urgently needed to treat the underlying diseases and block the accompanying cell damage that eventually leads to worsening of symptoms[8]. Protein kinases have become one of the important targets in drug discovery since the beginning of the 21st century, with marketing approvals for therapeutic applications, especially cancers [9]. However, compounds targeting protein kinases are still limited in other therapeutic areas, despite the crucial roles of these enzymes in various pathophysiological processes. Among such kinases, mitogen-activated protein kinase (MAPK) has attracted tremendous attention due to its roles in numerous cellular events, including differentiation, mitogenesis, cell survival and apoptosis [10]. Aim of this study was to screen out the effective bioactive compounds of *Cordia macleodii* which may be potential inhibitors of MAPK-signaling receptor in

future and may act as a drug which may be effective in preventing the various chronic diseases by blocking to MAPK receptor related signaling pathway.

II. MATERIALS AND METHODS

Molecular study was performed using different modules of bio-informatics tools. The schematic representation describes the work flow of the study Figure 1 followed by detailed description in the subsequent sections.

Modeling platform

All computational analysis was carried out on bio-informatics tools & related software version (AUTODOCK6.0) docking, grid generation, free energy calculations, and MD simulations). This software package programmed on DELL PRECISSION T1700 workstation machine running on Intel (R) Core (TM) i5-4590 CPU processor with 8GB RAM and 2 TB hard disk with centos WINDOW as the operating system. The schematic representation describes the work flow of the study followed by detailed description in the subsequent sections.

Biological data

In this study bioactive molecules were selected against the target of MAPKs. These bioactive molecules names and their physical characteristics were listed in Table 1 later, this collected bioactive molecules were retrieved from the chemical database and draw with help of *ChemSketch* (ACDlabs).[11] The (MAPKs) receptor was obtained from Protein Data Bank PDB ID: 30Z6.

Preprocessing and preparation of protein target structure

Protein X-ray crystal structures of MAPKs was obtained from the Protein Data Bank after converted into PDB format with the help of RosMol software. The protein preparation is using by the tool of protein preparation wizard on PyMol suite[12]. In general, protein is commonly occupied the water molecules. But, this process was evacuating those water molecules for increasing the entropy of target.[13,14]

Preprocessing and preparation of ligands

All the ligand molecules are prepared by the tool ACD labs .[15] Later these ligand molecules optimized on various ionization states, tautomer, stereo chemistries and ring conformations to adding molecules. It was using ligand rotatable bonds can move freely on further process.16,17

Molecular docking analysis

The PyRx (Autodock 6.0) was used to perform molecular docking and utilized to prepare the input .pdb file MAPKs (PDB ID:30Z6). Molecular docking uses the computational simulation predicts the ligand preferred orientation to a receptor when interact each other to form a stability complex. In this study PyRx version tool was used to perform rigid flexible docking for predicting binding affinity, ligand efficiency and inhibitory constant. Glide Extra precision (XP) tool is used for the justification of suitable ligand molecule to the active site of specific target. The ligands being docked were kept flexible.[18,19]

Molecular dynamics simulations

MD simulation was performed using ArgusLab and Discovery studio 3.1 version .41 The CHARMS in force field was used for the energy calculation. Constant temperature was 300 K and in the integration step 1.0 fs was given. Run the MD simulations for complex structure. MD simulation with position restraints was carried out for a period of 4000 PS to allow the accommodation of the water molecules in the system. Finally, Root Mean Square Deviation (RMSD) was calculated for checking the stability of 1KDM protein with their native motion. All the coordinate file was saved every 1000 ps upto 4 ns and the result was concluded by percentage lower and upper pose.[20-22]

Estimation of ligand binding energy using PyRx-Autodock

The ligand binding energy of two phytocompounds to inhibit MAPKs was estimated using PyRx module in .[23 The total free energy of binding, Δ G bind (kcal/mol) is estimated by the software as:

ΔG bind = G complex – (G protein + G ligand)

Where in each energy term is a combination of G = MME (molecular mechanics energies) + GSGB (SGB solvation model for polar solvation) + GNP (nonpolar solvation) Coulomb energy, Covalent binding energy, Van der Waals energy, Lipophilic energy, Generalized Born electrostatic solvation energy, Prime energy, Hydrogen-bonding energy, Pi-pi packing energy, Self-contact correcti]on.46We then used this score to rank the ligand-protein glide XP docked complex.[24]

III. RESULTS

Molecular docking

In this study, we intended to examine the overlaps MAPKs inhibitory effects of Cordia phytocompounds. In this protein sequence length is 377 amino acids (43.24kDa) and the resolution is 2.35 Å. These structures were to abolish water elements. A descriptive hydrogen atom was added to every inhibitor to assure that all of them were all-atom structures followed by energy minimization. After the protein preparation process is over, the protein is ready going with molecular docking. This molecular docking analysis has shown drug molecules potential and their hydrogen bond interaction where from the binding site of target. A two potent phyto- compounds molecules (phenol base compounds) in complex with MAPKs protein were docked. Each ligand was docked with MAPKs receptor that ligand molecules were produced docking score (binding affinity ΔG=-kcal/mol). The H-bond distance and their consequent glide energy were generated. And leading the docking score better drug for target molecules.50 Based on the research finding which molecule is placed leading docking score with the good binding affinities. We have strongly justified, it is a suitable ligand for target. In this examination, a natural compound of ***Cordia macleodii*** has shown better results than other molecules. On the other hand, Cordia phytochemicals is a flavonoid/phenolic nature. Moreover, this molecule is solving the ROS related problems and it was tested in both in-vitro and in-vivo.51 This analysis outcome cordia-2 compounds have received the docking score more than -5.8 Table 4. But, Cordia-1 acid is received maximum value of docking score-7.9 Table 4.

Molecular interactions of Cordia phytocompounds with functionally important residues of MAPKs

The MAPKs protein interactions with ligands surfaces are controlled by a complex array of intermolecular interaction. Such interactions depend both on the specific interactions in the binding site as well as the nonspecific forces outside the binding pocket. The protein- ligand interaction pattern between MAPKs and phytocompounds was analysis the site to which phytocompounds was binding. The Cordia-1 was robustly interacting with diverse residues of the hydrogen bond (Side-chain, Back-chain) HIS 169, THR 176, ASP 172, and ARG 241. In this interaction THR 176 residues is involved in two times and the ALA 236 also interact n-cation Figure 4. Analysis of docking results the results of our docking analysis, pertaining to each ligand is presented below. The docking scores and binding affinities are presented in Table 4. Cordia-1 through our molecular docking experiment, we found that cordia-1 efficiency. As a result cordia-1 had the best Glide G score (-7.9 kcal/mol) and RMSD lower pose (-42.01%) upper pose (60.81%) and number of pose nine. Analysis of the docked complex showed that the residues lys 134, Gly250, Ala 302 and Asp 168 (2) were involved in hydrogen bonding with Cordia-1 phytocompound. The residue Lys 134 was involved in hydration site with the ligand Figure 3a. cordia-2 had the Glide G score (-5.8 kcal/mol) and RMSD lower pose (-60.03%) ,upper RMSD pose (45.88%) with no pose created nine. . Analysis of the docked complex showed that the residues Thr 175 and Asp131 were involved in hydrogen bonding with Figure 4(2D pocket diagram.

Molecular dynamics simulations

The molecular dynamics simulation was carried out for the protein MAPKs and Cordia phytochemicals. For evaluate the structural constancy of those molecules with the help of Arguslab. The final trajectory files were taken for calculating the RMSD of the complex structures. At the same time as running MD simulation for MAPKs protein and Cordia phytocompounds for 10 ns, the RMSD (Root Mean Square Deviation) score in percentages shows the stability of the complex structures. The period and the constant potential energy stable at 1.2 ns to 10 ns. In addition, when performing the simulation for 10 ns, and it makes the stability of the complex structure during the entire simulation time up to 10 ns. The Cordia phytochemicals pharmacophore and energy minimization simulation score of Geometry and uv SCF score in table no.3 . The Esp mapping in imaged Figure 3.

IV. DISCUSSION

MAPK signaling pathways have been implicated in the pathogenesis of a variety of human disorders including cancer and neurodegenerative diseases such as AD, PD, and ALS. In AD, activation of MAPK cascades contributes to disease progression through regulation of neuronal apoptosis, β- and γ-secretase activity, and phosphorylation of APP and tau. Inhibitors for ERK1/2, MEK, or JNK, all of which contribute to the pathological hyperphosphorylation of tau, have been widely investigated as potential therapeutic drugs for AD [25,26]. Inhibitors of p38 MAPK are also considered as potential drugs for AD, given that the p38 pathway plays a key role in the Aβ42-induced production of pro-inflammatory cytokines [27]. MLK isoform-specific inhibitors such as CEP-5104 and CEP-6331 have been investigated in the mouse MPTP model of PD in an attempt to develop second-generation drugs based on the pan-MLK inhibitor CEP-1347 [28]. Aberrant expression and activation of p38 MAPK have been demonstrated in motor neurons and microglia of ALS patients. Several compounds including p38 inhibitors are under investigation as potential therapeutic agents

against ALS [29]. The ERK signaling pathway plays a central role in several steps of cancer development, including cancer cell migration and the development of resistance to apoptosis, such as that mediated by phosphorylation and consequent stabilization of the anti-apoptotic protein MCL-1. Inhibitors of the ERK signaling pathway are thus good candidates for the development of anticancer agents [30]. MAPK signaling pathways are also associated with the pathogenesis of several other chronic and genetic diseases such as Crohn's disease, polycystic kidney disease, and the Ras-MAPK syndromes. A clinical trial of CNI-1493, a JNK and p38 inhibitor, revealed beneficial effects on ulcer healing in patients with Crohn's disease, a chronic inflammatory bowel condition [31]. Polycystic kidney disease actually comprises a large family of genetic diseases characterized by renal failure. The Ras-MAPK signaling pathway has been implicated in the pathogenesis of polycystic kidney disease in studies with transgenic mice expressing H-Ras [32]. Germline mutation of the HRas gene has also been identified in Costello syndrome. Furthermore, dysregulation of the Ras-MAPK signaling pathway has been identified as a principal cause of the Ras-MAPK syndromes, which include Noonan, LEOPARD, Costello, and cardio-facio-cutaneous syndromes as well as neurofibromatosis type I [33]. Given that the same components of MAPK signaling pathways act differentially in the pathogenic mechanisms of many human diseases, knowledge of the tissue- and disease-specific regulatory mechanisms for MAPK signaling pathways might provide clues for the development of new therapeutic drugs for human diseases. Earlier, many disease-causing receptor proteins to predicting various bioactive molecules respectively [34,35]. End of the outcome validation all the phytocompounds were validated by the binding mode of the target. The suitable ligand molecules have filtered based on the binding affinities of ligand to target amino acid residues. Binding affinities shows the contribution of ligand from target and strongly rely on the flexibility of receptor.

V. CONCLUSION

As a result of this computational experiment Phytocompound of the Cordia-1(4 5)-1formy 1-4-dihydroxy-3oxo 3-4 dihydro-2H-pyran-1-ium) has shown efficient docking score and effective binding affinities. Hence, we concluded that the Cordia-1 may be a suitable potential to the MAPKs stimulation. Based on this finding, we suggested that Cordia-1 bioactive molecule used for further drug development process. And, this study will be addressed to further drug processing analysis.

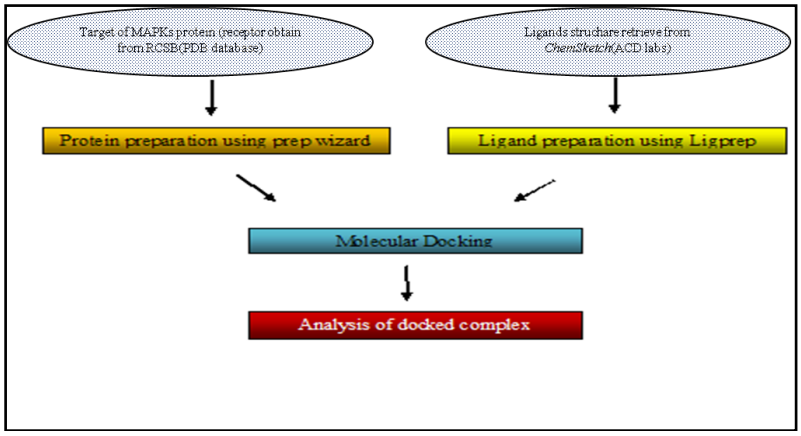

Figure 1 Schematic representation of the docking procedure and analysis

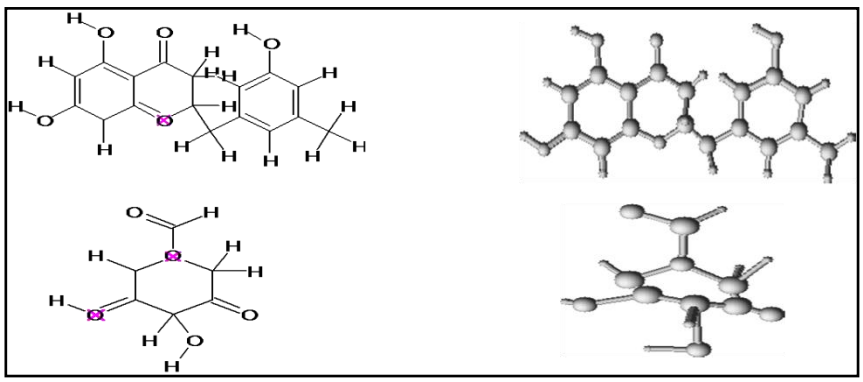

Figure 2: Bioactive molecules and their 2-D, 3-D structure of ***Cordia macleodii***.

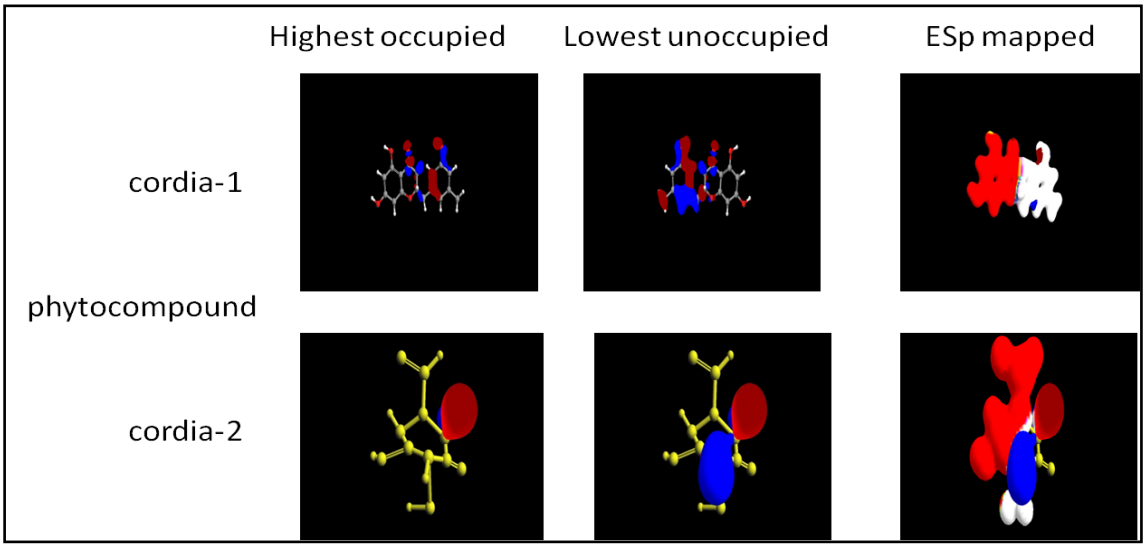

Figure 3: Energy simulation and pharmacophore estimation with the help of ***Arguslab***

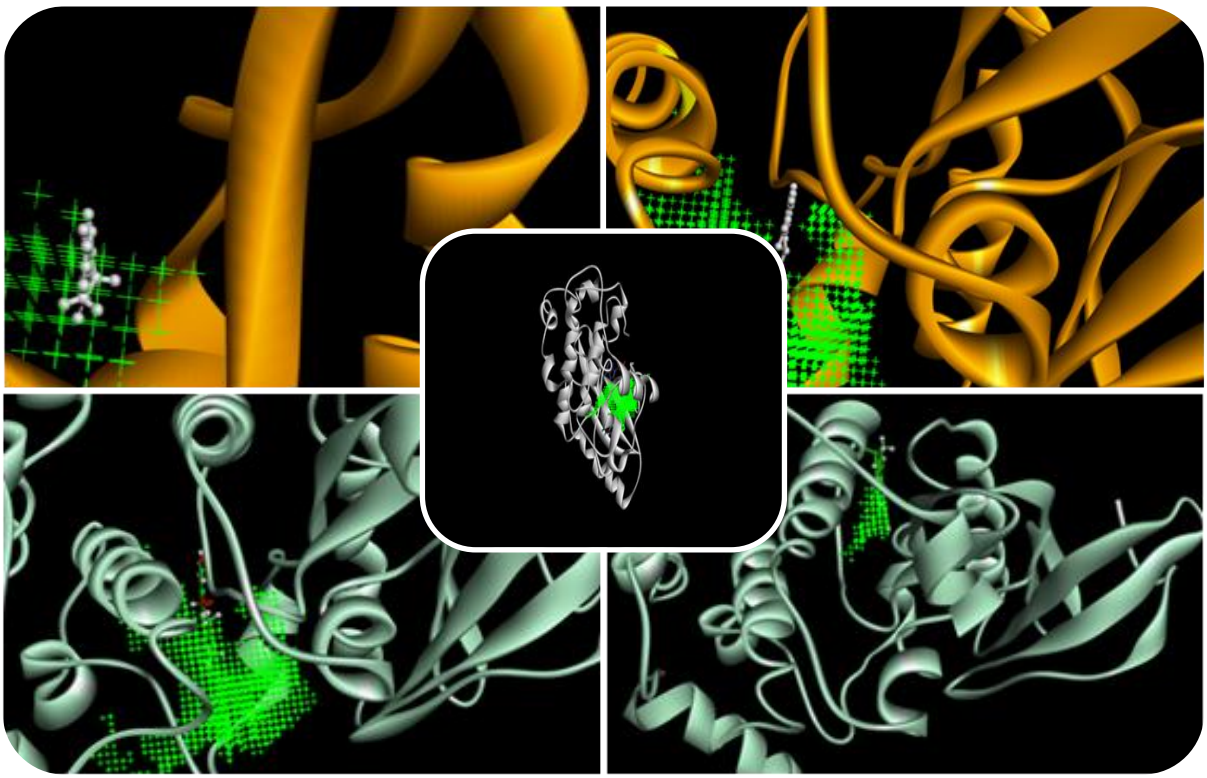

Figure 4: Docked complex of 30Z6 and phytocompounds of Cordia. Dashed grey line indicated hydrogen interaction n between target residues as well as ligand. (b). 2-D Structural view; represented hydrogen bond back chain and blue dotted lines represented hydrogen bond side chain. Red color indicated that ligand salt bridge interaction.

Table 1: ***Cordia macleodii*** phytocompounds bioactive molecules IUPAC name and their physical characteristics

S.NO.	Properties	Phytocompounds	
		Cardia -1	Cardia-2
	Name of chemical	(4 5)-1formy 1-4-dihydroxy-3oxo 3-4 dihydro-2H-pyran-1-ium	5,7-dihydroxy-2{3-hydroxy-5methyl phenyl] 2-3 dihydroxy -4H benzopyran
1	Molecular formula	$C_6H_7O_5$	$C_{17}H_{16}O_5$ s

2	Molecular weight	159.1162314	300.30594
3	composition	-	-
4	Molar reactivity	34.03	81.34

Table 2: Extra Precision Glide docking results with interacting amino acids in the active of SHBG

. S. no.	Specification	Characterization
1	PDB ID	30Z6
2	Molecular size	51681.58
2	Solubility	Hydrophobic
4	Chain	A,B
3	Length	388
6	Amino acids	377
4	Type	Structural
5	Total Structure Weight:	43.24(kDa)

Table 3: Extra Precision Glide docking results with interacting amino acids in the active of SHBG

S.NO	Specification	Cordia 1	Cordia2
1	SCF energy	-27.1905324951	-127.8090524794
2	Geometry	-59.893585417	-146.938338
3	UV		-167.8905632757

Table 4. Mean values of docking energies (kcal/mol) and standard deviation for each skeletal type of Cordia macleodii phytochemicals as ligand with anti-oxidant enzymes enzyme targets.

Target	ligand	Dimension Centre(x=25Ay=25z=25	No of pose	RSD %lower	RSD %upper	Mean binding energy
MAPKs	Cordia-1	X=7.3512,y=47.6457, z=0.0769	9	42.01%	60.81%	-7.9
	Cordia-2		9	60.03%	45.88%	-5.8

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