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AN *INSILICO* STUDY TO IDENTIFY POSSIBLE NOVEL MITOGEN ACTIVATED PROTEIN KINASES SIGNALLING PATHWAY INHIBITORS FROM SELECTED NATURAL ANTIOXIDANTS.

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ABSTRACT

Prolonged exposure to stress induces changes in various neural circuits of different brain regions that alter the balances in neuronal activities involving microglial activation, signalling pathways and cellular homeostasis by antioxidants. Sustained and extended activation of Mitogen activated protein kinases (MAPK) signalling cascades, release proinflammatory cytokines leads to neuroinflammation and neurodegeneration. As various phosphorylated MAPK subfamilies such as P38MAPK, c-Jun N terminal kinase (JNK), Extracellular receptor kinases (ERK) ½ and ERK5 are known to be involved in this pathway and the inhibitors of the pathway become the targets for developing efficient drugs in the drug discovery field. In the current study, the antioxidants of natural origin present in the plants namely α asarone, Quercertin, Diosmetin, Resveratrol, Glabridin, were used to identify efficient inhibitor against MAPK's pathways. In this study, an insilico approach was done to analyse the drug like properties of the ligands. DruliTo and ADMET Sar softwares were employed in the analysis of the following profiles- Blood brain barrier permeability, Human intestinal absorption, toxicity profile and LD50 values. Molecular docking was done by iGEMDOCK software. The results of IGEMDOCK shows that except Resveratrol all other four ligands namely α asarone, Quercertin, Diosmetin, Glabridin has got lower binding energy, good ADMET sar profile and also all the drugs obey's the lipinski's rule. The result of this study indicates that the antioxidant extracts of α asarone, Quercertin, Diosmetin, Glabridin can be tried as a novel inhibitor of MAPK signalling pathways. Further *in vivo* and *in vitro* studies are necessary to validate and implement these drugs in clinical trials.

KEYWORDS – **MAPK, *Insilico*, Antioxidants, Drulito, ADMET Sar, iGEMDOCK**

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INTRODUCTION

Exposure to various stresses causes continuous release of oxygen free radicals from the cell and to neutralizing them, the antioxidants must be produced in balanced amount as without antioxidant defence there occurs neuronal damage in relation with neurodegenerative diseases.[1] A wide range of cellular stressors activate Mitogen-activated protein kinases(MAPK) which are a specific class of serine/threonine kinases. The MAPK subfamilies are Extracellular signal related kinases (ERK1/2 & ERK5), c-Junction N terminal kinases (JNKs) and p38MAPK.[2] Neurodegenerative diseases is a combination of progressive increase in neuronal loss and dysfunction of these MAPK signalling pathways can be linked to the pathogenesis of various neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, Amyotropic lateral sclerosis, Multiplesclerosis, Huntington's diseases.[3] Various animal studies have shown that p38MAPK and its isoforms are localised in several areas of brain mainly in the hippocampus.[4,5]Not only in neurodegenerative diseases but also the p38 MAPK and its isoforms are predominantly activated in inflammatory diseases like Rheumatoid arthritis, Osteoarthritis.[6]MAPK signalling cascade activation has been seen in rats exposed to cold immobilization stress leading to mucosal inflammation and gastric injury.[7] c-junction N terminal kinases (JNKs), one of the MAPKinases are involved in neuroinflammatory process in various inflammatory diseases.[8] Extracellular signal related kinases namely ERK1/2 have been shown to be important in suppressing the learning, memory and dysregulation of limbic circuit in stress related disorders. Corticotropin releasing hormone mediated phosphorylation of ERK1/2 in the pyramidal cell layer of hippocampus was observed in stressed rats. [9] During any stressful condition microglial cells are activated and there is release of proinflammatory cytokines. Initially they have been assigned for protective mechanism. Prolonged release of proinflammatory cytokines can cause neuronal cell death due to activation of MAPK signalling cascade.[5] Thus the role of MAPK signalling pathway in the generation of oxygen free radicals are well established. In various neurodegenerative disease the release of reactive oxygen species causes damage like nucleic acid breakage, enzyme inactivation, polysaccharides depolymerisation and other destruction leading to cell death.[10] There are natural compounds available which are shown to have antioxidant effects can be used as counter these damages. The active principle -α asarone, from the plant, *Acorous calamus linn*; and another substance, Glabridin, from the plant *Glycyrrizha glabra*, has been reported to have antioxidant effect which can counteract oxygen free radicals.[11,12] Resveratrol (3,4,5- trihydroxy-trans-stilbene), Quercetin (2-3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one),Diosmetin,(5,7-dihydroxy-2-(3-hydroxy-4 methoxyphenyl)chromen-4-one) all the above mentioned compounds are polyphenols and flavanoids from natural source that have been proved to have antioxidant and MAPK inhibitory activity when administered against various disease like cancer, Alzheimer's [13,14,15]Elucidating the role of inhibitors of MAPK signalling pathway or cascade may help to find a novel therapeutic drug so as to slow down the pathogenesis and to treat various diseases. Structure based drug design or drug target docking by computational methods are time and resource saving processes by which an efficient and effective therapeutic drug can be designed or discovered.[16] The effective absorption, distribution, metabolism, excretion (ADME) and toxicity profiles are measured by *insilico* method so that a novel drug can be designed which is safer for therapeutic purpose after clinical trials.[17] Hence in our study we focus on finding novel therapeutic MAPK inhibitor from the natural polyphenolic compound like Resveratrol, Quercetin, Diosmetin, α asarone from the plant *Acorous calamus linn* and Glabridin from the plant *Glycyrrizha glabra* by using computational method.

MATERIALS AND METHODS

Preparation of targets

The PDB structure of target was gained from RCSB The PDB structures of the targets was visualized in Rasmol (Table:1)

Table1

Data & Databases of Target

S.No	Data	Database
1.	p38	3GCV
2.	JNK	1JNK
3.	ERK1/2	1TVO
4.	ERK5	2Q8Y

Ligand datasheets and optimization of ligand

Table 2

IUPAC Names, Pub Chem Id, Molecular Formula of 5 Ligands

S. No	IUPAC names	Molecular formula	Pub Chem. ID
1.	Resveratrol – (3,4,5-trihydroxy-trans-stilbene)	$C_{14}H_{12}O_3$	ID – 445154
2.	Quercetin - (2-3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one)	$C_{15}H_{10}O_7$	ID – 5280343
3.	Diosmetin – (5,7-dihydroxy-2-(3-hydroxy-4 methoxyphenyl)chromen-4-one)	$C_{16}H_{12}O_6$	ID – 5281612
4.	Alpha asarone - (1,2,4-trimethoxy-5-[(E)-prop-1-enyl]benzene)	$C_{12}H_{16}O_3$	ID – 636822
5.	Glabridin - 4-[(3R)-8,8-dimethyl-3,4-dihydro-2H-pyrano[2,3-f]chromen -3-yl]benzene -1,3-diol	$C_{20}H_{20}O_4$	ID – 124052

The molecular structures of the ligands used in the current study (natural antioxidants) were downloaded from Pub chem. Database in SDF format. IUPAC names and the pub chem. ID's of the selected five ligands are Shown in Table 2. Later the 2D structures of these compounds were converted by Open Babel software as MDL format.(www.vcclab.org/lab/babel/start.html) Molecular docking with iGEMDOCK:-iGEMDOCK is used in the current study for docking and virtual screening of the target- ligand interaction profile as this docking tool is more reliable and accurate in drug discovery field.[18]Rapid virtual screening of the ligands in iGEMDOCK V2.0 with a population size of 800 is set with 80 generation and 10 solution for accurate docking. It is used because iGEMDOCK showed better performance as compared with other docking softwares as the results are predicted based on the interaction profiles like Electrostatic energy, Vander waal energy, Hydrogen bond energy, Elect.[19,20]After completion of docking post screening analysis with pharmacological interactions was done as it is an important and key step in docking related studies. The post analysis were done based on the "Lipinski's" rule of five and other drug like properties using ADME Sar software for analysis of absorption, distribution, metabolism, excretion and toxicity profile of the five ligands.The selection of the best among five ligands was based on the Lipinski rule and ADMET properties. The Lipinski's Rule of five (or) Lipinski alert Index predicts the drug properties as follows.

If a compound has

- H- bond donors is less than 5
- H- bond acceptors is less than 10
- Molecular weight is less than 500 daltons
- Moriguchi's lop P is less than 5

then the probability of using the compound as therapeutic drug is more.[21,22]

RESULT AND DISCUSSION

In the current study, the efficiency of the drug likeliness and Pharmacological properties of the chosen ligands (α asarone, Glabridin, Resveratrol, Diosmetin and Quercertin) on the four targets (p38, JNK ERK1/2, ERK5) binding sites were analysed computationally. As the first step the four targets namely 1) p38 2) JNK 3)ERK1/2 4) ERK5 were downloaded from RCSB. The second step was downloading the ligands from Pubchem databases and the drug likeness scoring was done using Drulito and ADMET sar softwares.ADMET Sar predicts the regression values of various ligands were shown in Table -3. The different routes of entry like Blood brain barrier (BBB), Human Intestinal absorption (HIA), $CaCo_2$ permeability, P-glycoprotein efflux (p-gp) substrate/ inhibitor were tested.[23]

Table 3
The Result of ADMET Predicted Profile of the five ligands

S. No	Ligands Name	BBB	HIA	CYP inhibition/ Substrate	AMES Toxicity	Carcinogenicity	LD50 in rats
1	α asarone	0.9151	1.0000	Non-substrate/ Non inihibitor	Non toxic	Noncarcinogenic	1.9737
2	Diosmetin	0.6382	0.9783	Non-substrate/ Non inihibitor	Non toxic	Noncarcinogenic	2.7192
3	Glabridin	0.5803	0.9892	substrate/ Non inihibitor	Non toxic	Noncarcinogenic	2.9435
4	Quercetin	0.5711	0.9650	Non-substrate/ Non inihibitor	Non toxic	Noncarcinogenic	3.0200
5	Resveratorl	0.5900	0.9952	Non-substrate/ Non inihibitor	Non toxic	Noncarcinogenic	1.6791

The main strategy in drug delivery is its ability to cross the BBB, so that it can inhibit or act on the target particularly in brain. The transmembrane diffusion of the drug depends upon the physiochemical properties of the drug.[24] The maximum penetration of the drug through blood brain barrier depends upon the highest probability value in ADMET sar profile and the penetration of the drug α-asarone is predicted to be higher of about 0.9151 in crossing the BBB. Analysing the substrate/inhibitor profile of the P-glycoprotein (P.gp) is a energy dependent efflux pump present in BBB and is responsible for efflux system. To choose a substrate as better ligand, the compound should be noninhibitor of CYP450 so that the biotransformation of drug metabolized by CYP450 microsomal enzymes will not be halted. This study showed that the compounds Disometin, Glabridin, Quercetin was predicted as substrate and non inhibitor whereas for α-asarone & Resveratrol comes out to be nonsubstrate and non inhibitor. The ADMET sar profile showed positive and no side effect on absorption as the HIA score of all ligands were higher and the score of α-asarone is highest of 1.0000. Further, the results of DruLiTo software clearly showed that all the five natural compounds chosen for the study obeyed the lipinski's rule (Table-4) and so passed the eligibility test to be used as therapeutic drugs.

Table 4
The Result of the Lipinski's Properties of the Five Ligands

S.no	Ligands Name	Molecular Weight	xLog p	H Bond Acceptor	H Bond Donor	TSPA
1	α asarone	324.14	0.981	4	2	58.92
2	Diosmetin	300.06	-0.723	6	3	96.22
3	Glabridin	228.08	1.194	3	3	60.9
4	Quercetin	208.11	0.619	3	0	27.69
5	Resveratorl	302.04	-1.244	7	5	127.45

The IGEMDOCK generates the results about target-ligand interaction profiles like electrostatic energy (Elec) , hydrogen binding (Hbond), vanderwaals energy(vdW) and total binding energy. Here, lower the binding energy, more promising the compound to act as a therapeutic drug. The post docking analysis of the five natural compounds with four substrates targets of MAPK's are shown in Tables 5a-d.

Table 5a
The Results of IGEMDOCK showing the Binding Energies of Five selected Ligands with the target P38 MAPK

S.No	Ligands	Total Binding energy (kcal/mol)	Vander waals force	H bond
1.	Alpha asarone	-116.772	-103.49	-13.3
2.	Disometin	-127.346	-115.01	-12.3
3.	Glabridin	-123.767	-116.80	-6.9
4.	Quercetin	-117.76	-103.71	-14.0
5.	Resveratorl	-88.0546	-75.88	-12.1

Table 5b
The results of IGEMDOCK showing the Binding energies of five Selected Ligands with the target JNK

S.No	Ligands	Total Binding energy (kcal/mol)	Vander waals force	H bond
1.	Alpha asarone	-97.5726	-91.13	-6.4
2.	Disometin	-117.023	-113.5	-3.4
3.	Glabridin	-94.3102	-91.81	-2.5
4.	Quercetin	-101.62	-98.11	-3.5
5.	Resveratorl	-70.3154	-68.74	-1.5

Table 5c
The Results of IGEMDOCK showing the Binding energies of Five selected ligands with the target ERK ½

S.No	Ligands	Total Binding energy (kcal/mol)	Vander waals force	H bond
1.	Alpha asarone	-102.075	-77.84	-24.2
2.	Disometin	-102.556	-93.74	-8.85
3.	Glabridin	-101.826	-91.74	-10.0
4.	Quercetin	-110.943	-94.85	-16.0
5.	Resveratol	-73.9147	-68.85	-5.05

Table 5d
The Results of IGEMDOCK showing the Binding energies of five selected ligands with the target ERK 5

	Ligands	Total Binding energy (kcal/mol)	Vander waals force	H bond
1.	Alpha asarone	-126.301	-80.0	-29.3
2.	Disometin	-109.369	-78.1	-48.1
3.	Glabridin	-84.4817	-78.4	-5.9
4.	Quercetin	-109.356	-80.1	-29.2
5.	Resveratol	-83.3482	-62.7	-20.6

The iGEMDOCK result of the ligands with the target P38 MAPK showed among the given ligands, the Diosmetin and Glabridin become promising as therapeutic drugs as the binding energy of Diosmetin, Glabridin with the target P38 MAPK was -127.35 kcal/mol & -123.77 kcal/mol respectively, which is the lowest when compared to other ligands. Further, ADMET sar analysis revealed that the Diosmetin and Glabridin also have the property to cross the BBB as their respective score was 0.6382 and 0.5803 which was good score though not as good as the same score of α-asarone (0.9151).The intestinal absorption score for diosmetin is 0.9783 and that of Glabridin is 0.9892 overall absorption is good for both ligands. The ligands are non substrate and noninhibitor, hence the compound can be metabolized by cytochrome P450 enzymes - no hepatotoxicity The results suggest that these two ligands will be good inhibitors of p38 MAPK signalling pathway. The better Docking pose of Diometin and Glabridin in iGEMDOCK is shown in Figure-1and 2.

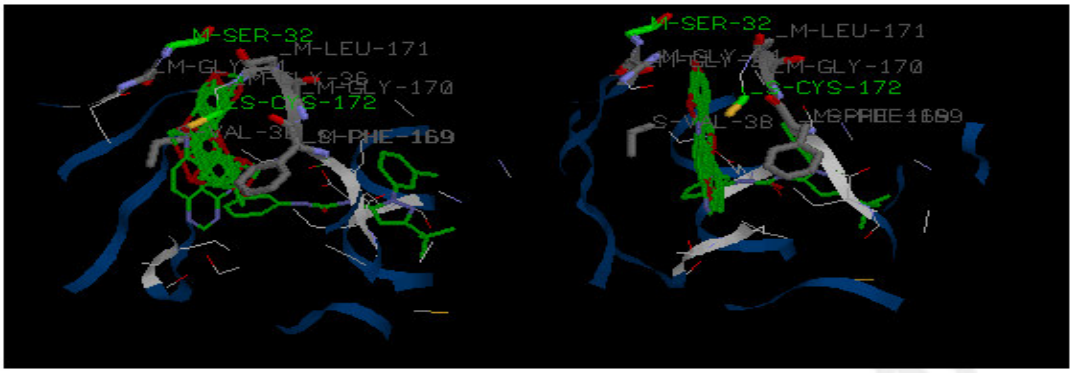

Figure 1 and 2
Docking Poses of Disometin and Glabridin With P38 MAPK

The iGEMDOCK results of JNK targets (Table-5b & fig 3) clearly showed that only the ligand Diometin has lower binding energy of -117.02 and so become better inhibitor of JNK pathway. It also had good absorption score, no hepatotoxicity, was non carcinogenic and nontoxic with LD50 of 2.7192 with no oral toxicity.

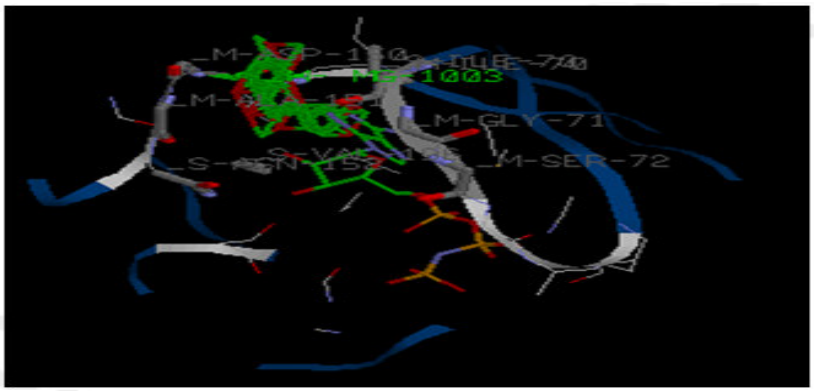

Figure 3

Docking pose of Disometin with JNK

On the other hand, the iGEMDOCK result of the target ERK ½ (Table -4) preferred the ligand Quercetin as its binding energy of -110.95 kcal/mol. The result with the target ERK ½ showed that all the ligands had good binding energy with ERK½ except Resveratorl. The ADMET sar result showed that the drug Quercetin can cross the BBB, can be absorbed in the intestine and is non inhibitor of cytochrome P450 hence will not produce hepatic toxicity. It is also safer for oral admistration for rats as it has got the highest LD50 of 3.0200 when compared other drugs. The better docking pose is shown in Figure -4

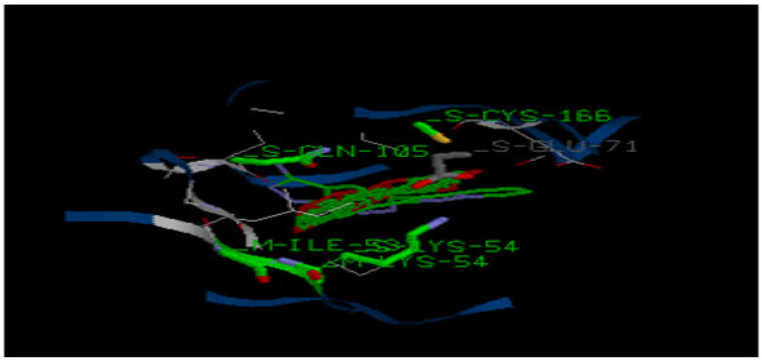

Figure 4

Docking pose of Quercetin with ERK1/2

Finally, the analysis of Target-Ligand interaction profile of ERK5 (Figure-5)indicating that the promising inhibitor could be α-asarone as its binding energy is -126.30 kcal/mol. The drug α-asarone has got BBB score of 0.9151 which is highest of all the four drugs. This shows that the drug can cross the BBB more effectively. It also has good intestinal absorption score(Table -2).non toxic, noncarcinogenic, and no hepatotoxicity.

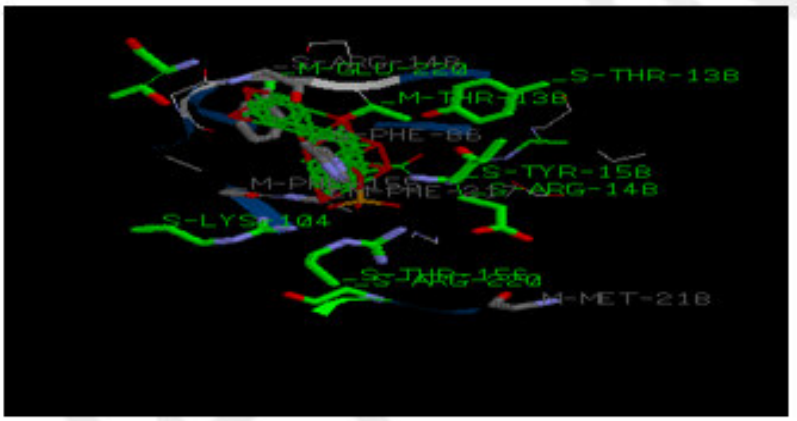

Figure 5

Docking pose of α asarone with ERK 5

CONCLUSION

Our current study results shows that the compound α-asarone is a good inhibitor of ERK5 pathway, Quercetin, a promising inhibitor of ERK1/2 signalling cascade, Diosmetin a good inhibitor of JNK pathway and P38 MAPK along with Glabridin. These MAPK signalling pathways are phosphorylated during various stressors, creating a better inhibitors of these pathways will help in halting the pathogenesis of various neurodegenerative or neuroinflammatory diseases. Further *in vitro* and *in vivo* animal researches are necessary for clinical trials or therapeutic use of these drugs.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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