INTRODUCTION

Mitogen-activated protein kinase 14 (MAPK 14), also called p38α MAPK, is an enzyme that in humans is encoded by the MAPK 14 gene [1]. MAPK 14 gene encodes p38α MAPK which is the prototypic member of the p38 MAPK family. p38 MAPKs are also known as stress-activated serine/threonine-specific kinases. In addition p38α, the p38 MAPK family has three additional members' p38β, p38γ, and p38δ isofoms, encoded by MAPK 11, MAPK 12, and MAPK 13 genes, respectively. p38α MAPK was originally identified as a tyrosine phosphorylated protein detected in activated immune cell macromolecules with an essential role in inflammatory cytokine induction, such as tumor necrotic factor α (TNF-α) [2,3]. However, p38α MAPK is ubiquitously expressed in many cell types [4,5] and p38α mediated kinase activity has been implicated in many functions beyond immune system. Pharmacological and genetic inhibition of p38α not only revealed its biological significance in physiological function but also the potential of targeting p38α in human diseases such as autoimmune diseases, heart failure, Alzheimer disease, and Parkinsonism.

Role of p38α MAPK in heart failure

p38α MAPK activity is implicated in cardiac hypertrophy which is a significant feature of pathological remodeling in the diseased hearts and a major risk factor for heart failure and adverse outcomes [6-9]. Chronic activation of p38α MAPK activity is viewed as pathological and pro-apoptotic, and its inhibition is in clinical evaluation as potential therapy to mitigate acute ischemic injury in ischemic heart failure and against reperfusion injury after revascularization [10-12].

Role of p38α MAPK in Alzheimer’s disease and Parkinsonism

Alzheimer’s disease is the most common form of dementia and is becoming increasingly prevalent with an estimation that 1 in 85 people globally will be affected by 2050 [13]. The disease is typically characterized by the presence of Aβ plaques or neurofibrillary tangles (NFTs) formed from free aggregated neuronal microtubule-associated protein “tau” within the brain. Tau has been shown to be phosphorylated by p38α MAPK in neurons. Tau is a highly soluble microtubule-associated protein in which sub-cellular localization is determined by its phosphorylation status in neuronal cells. The principal function of tau is to bind and stabilize cytoskeleton microtubules (MTs). The ability of tau to interact with and stabilize MT is regulated through post-translational modifications, mainly through phosphorylation of serine and threonine residues. This regulatory phosphorylation is tightly controlled by numerous protein phosphatases and kinases, and consequently by p38α MAPK [13,14]. The phosphorylation of particular residues Ser-356, Thr-231 and Ser-212 has been proposed to instigate detachment of tau from the MT, and since these residues has been shown to be phosphorylated by p38α MAPK, it could potentially be involved in tau detachment from MT and thereby destabilization of the MT [14-18]. Physiological role of p38α MAPK in tau phosphorylation can turn into a pathophysiological role if tau becomes hyperphosphorylated, as this hyperphosphorylation results in increased tau detachment from MT then there is an increase in the amount of soluble tau present in the neuron, which is therefore prone to self-aggregation and polymerization, leading to the formation of tau oligomers. These oligomers combine and further aggregate to form paired helical filaments which then assemble to form Amyloid containing plaques or NFTs as seen in Alzheimer’s disease. In addition to the aforementioned association with plaques and tangles, p38 MAPK is involved with the inflammatory response. It was shown that Aβ amyloid is able to stimulate glial cell cultures and activate p38α MAPK [19], thus upregulating the production of inflammatory enzymes and cytokines which contribute to the inflammatory response.
cytokines such as interleukin-6 (IL-6), IL-1β and TNF-α [20,21]. It is this increased release of inflammatory mediators from overstimulation of NF-κB-stimulated glial cells that can cause a neuroinflammatory and neurotoxic effect on surrounding neurons contributing to the loss of neurons witnessed in neurodegenerative disease [22]. Similar injury to neurons by inflammation is also seen in Parkinson’s disease. α-Synuclein is the predominant fibrillar component of the proteinaceous Lewy bodies seen in Parkinson’s disease. α-Synuclein released from damaged neurons interacts with microglia and the activation of glial cells through the p38 MAPK consequently results in the production of inflammatory cytokines and TNF-α, which promote neuroinflammation [23].

**Role of p38 MAPK in inflammation**

Activation of MK2 (MAPK activated protein kinase-2) by p38α MAPK plays a pivotal role in upregulating the secretion of inflammatory cytokines, IL6, IL-1β and TNF-α by immune cells [24]. Hence, drugs targeting p38α MAPK have potential for autoimmune diseases like psoriasis, rheumatoid arthritis, etc. apart from neurodegenerative diseases and ischemic heart failure.

**Anti-inflammatory and neuroprotective actions of Morinda citrifolia**

The *M. citrifolia* plant is one of approximately 11,000 species of the family Rubiaceae. Furthermore, as a member of the mulberry family, it is a popular plant used for curative and preventive purposes in Polynesia as almost all of its parts in various combinations have been used to treat various known acute and chronic diseases in this region for 2000 years [25,26]. The anti-Alzheimer effect of an ethyl acetate extract of *M. citrifolia* fruits was studied by Murakishiar et al. in a mouse model. It was shown that the extract ameliorated beta-amyloid peptide-induced cognitive dysfunction in mice and there was a significant increase in short-term memory and long-term memory as observed by the step-down inhibitory avoidance behavior and significant decrease in escape latency was noticed in the animals treated with extract in the water maze [27]. Xu et al. showed that feeding of extract of *M. citrifolia* fruits to neonatal foals (newborn horses) decreased monocyte expression of TNF-α and cyclooxygenase-2 [28]. In BALB/c mouse model Hokama et al. showed that alcohol extract of *M. citrifolia* fruit at various concentrations inhibited the production of TNF-α, thereby inhibiting its tumor promoting effect [29].

Thus, various experimental evidences have shown that *M. citrifolia* has anti-inflammatory and anti-Alzheimer properties. The extract of *M. citrifolia* is a mixture of various phytochemicals.

The molecular mechanisms by which *M. citrifolia* extract exerts neuroprotection and inhibition of cytokine production have not been studied in detail so far. Currently, molecular docking approach has been used in modern drug design and to understand drug-receptor interactions. This paper reports screening of various phytochemicals present in *M. citrifolia* against p38α MAPK enzyme extracted from Protein Data Bank (PDB), by utilizing the Molegro virtual docker (MVD) Software [30].

**METHODS**

The structure of p38α MAPK (PDB ID 1DFW) was obtained from the PDB (http://www.rcsb.org). The structure contains ligands MW181 (C14H19N3O3, NN-dimethyl-6-(naphthalen-1-yl)-5-(pyridin-4-yl)pyridazin-3-amine or MW01-10-181SRM) and GGS (C14H19N2O4 [C16H17N2O4] + [2-x-fluorophenyl]-1H-pyrrozol-4-yl(pyridine) co-crystallized to p38α MAPK. In vitro and in vivo assays demonstrated that MW181 ameliorates beta-amyloid-induced synaptic and cognitive dysfunction. The active site targeting of the inhibitor was confirmed by high-resolution crystallographic analysis, and the structure has been deposited with RCSB-PDB to facilitate future kinase inhibitor design [31].

Literature search was done which revealed phytochemicals present in *M. citrifolia* fruit reported by various authors. Major iridoids present in *M. citrifolia* fruit are asperuloside and asperulosidic acid. Minor iridoids of *M. citrifolia* include dehydro-methoxy gaertneroside, epidihydropinitoside, 6'-alpha-hydroxyadroxyxioside, citrinulin-B, and 7-beta-epoxy-8-epi-splenodioside. Flavon glycosides present in the fruit are rutin, narinioside, and nicotifloroside. Several known and new lignans like 3,4'-bisdemethylpinoresinol, americanol-A, americanin, isoprinone, and balaphonin are also present. Miscellaneous compounds such as J-Sterostrol and its 3'-glucoside, ursolic acid, 19-hydroxysursolic acid, cytisine, borreijerin, epiborreijerin, 4-hydroxy-3-methoxycinnamaldehyde, hydroxypropiovanillone and vanillin have been isolated from the fruit [32]. Vomforfol [33], astroctortin [34], pinosinol and queretin [35], rubidin and nordamnacanthol [36], monidine-5-O-methyl ether [37], are also present in *M. citrifolia* fruit.

Of the above 32 principle compounds present in fruit 22 molecules which satisfy at least 4 conditions of the Lipinsky 5 rule (molecular weight ≤500 g/mol, oil/water distribution coefficient (Log P) ≤5, hydrogen bond donors ≤5, hydrogen bond acceptor ≤10, number of rotatable bonds ≤10) [38] were selected for docking. The structure of these compounds was downloaded as SDF files from Pubchem compounds database. To make accurate predictions, it is important that the imported structures have been properly prepared, that is, the atom connectivity and bond orders are correct and partial atomic charges are assigned. SDF files often have the poor or missing assignment of explicit hydrogens. All necessary valence checks and H atom addition were thus performed using the utilities provided in MVD.

MVD automatically identifies potential binding sites (also referred as cavities or active sites) by using its cavity detection algorithm. The cavities centered at the experimentally known ligand position were used. In the case of the crystal structure of p38α MAPK, the program identified five different binding sites. From these five predicted cavities, Cavity 1 with the highest volume containing bound ligand MW181 and Cavity 2 containing bound ligand GG5 were selected for docking. Native ligands were extracted and redocked into appropriate active sites. The results yielded control parameters for comparing with *M. citrifolia* phytochemicals.Docked native ligands were viewed in workspace and were found to have optimal orientation.

Ten independent runs were performed for each ligand with the guided differential evolution algorithm, with each of these docking runs returning one solution (pose). The Moldock scoring function used by MVD is derived from the scoring functions originally proposed by Gehhaar et al. and extended later by Yang et al. [39]. The 10 solutions obtained from the 10 independent docking runs were re-ranked, to further increase the docking accuracy, using a more complex scoring function. The mean and standard deviation of the scores were calculated. The docking scores of *Morinda* phytochemicals in Cavity 1 and Cavity 2 were validated by comparing them with the docking scores of MW181 and GG5, respectively. In the studies reported here, MVD was used because it showed higher docking accuracy when benchmarked against other available docking programs (MD: 87%, Glide: 82%, Surfleex: 75%, FlexX: 58%) ([Figs. 1 and 2] [30]).

**RESULTS AND DISCUSSION**

**Cavity 1, reference ligand MW181**

Among 22 compounds docked, compound isoprinone (C27 H26 O9, moldock score = -1114.16,339 kcal/mol) showed better docking score, and rerank score compared to reference ligand MW181 (moldock score = -1112.9±8.20 kcal/mol) present in Cavity 1 in the crystal structure. Negative values indicate that the bond is more stable. Higher negativity means the bond is more stable and the interaction may hinder the performance of the enzyme (Table 1).

A recently emerging variant of the active site approach focuses on induction of localized conformational changes by inhibitor to achieve selectivity. In the case of p38α MAPK inhibitors, the approach is by exploiting hydrogen (H)-bond interactions with the Met109 amide bond in the phylogenetically conserved kinase hinge region.
that can also engage the contiguous Gly110, thereby inducing a localized conformational change, termed as glycine flip [40-43]. In our study isoprincepin showed similar H-bond interaction with Met 109 amide bond and hence can be expected to have high selectivity (Figs. 3 and 4).

**Cavity 2: Reference ligand GG5**

Isoprincepin and balanophonin (C_{20}H_{20}O_{6}) show better docking scores when compared to reference ligand GG5 in Cavity 2 (Table 2) and the compounds have hydrogen bond interaction with Ser 293 similar to GG5 (Figs. 5 and 6).

Lipinski also laid down rules derived from the set of 1500 drugs that were filtered for good blood-brain barrier penetration and brain penetration is likely if, molecular weight ≤400 g/mol, Log p≤5, hydrogen bond donor ≤3, hydrogen bond acceptor ≤7 [44].

### Table 1: Mean moldoc scores and re-rank scores of docked ligands of Cavity 1

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Moldoc score kcal/mol</th>
<th>Rerank score kcal/mol</th>
<th>Hydrogen bonds</th>
<th>Bonding amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW181</td>
<td>-111.29±8.20</td>
<td>-79.62±5.11</td>
<td>3</td>
<td>His 107, Lys-53</td>
</tr>
</tbody>
</table>

*Moldoc and rerank scores expressed as mean±standard deviation*

### Table 2: Mean moldoc scores and re-rank scores of the docked ligands of Cavity 2

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Moldoc score kcal/mol</th>
<th>Rerank score kcal/mol</th>
<th>Hydrogen bonds</th>
<th>Bonding amino acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG5</td>
<td>-120.31±7.36</td>
<td>-94.74±8.78</td>
<td>3</td>
<td>Ser-293</td>
</tr>
<tr>
<td>Balanonphonin</td>
<td>-135.27±3.41</td>
<td>-107.59±5.40</td>
<td>4</td>
<td>Ser-293, Ser-251, Ser-252</td>
</tr>
<tr>
<td>Isoprincepin</td>
<td>-177.52±6.22</td>
<td>-133.60±10.59</td>
<td>3</td>
<td>Ser-293, Ser-251, Ser-254</td>
</tr>
</tbody>
</table>

*Moldoc and rerank scores expressed as mean±standard deviation*
Based on physiochemical parameters balanophonin has good potential to cross blood brain barrier and to be a p38α MAPK inhibitors in brain (Table 3).

Blocking of p38α MAPK can alleviate inflammation in brain and periphery through cytokines and also Tau hyperphosphorylation and thereby amyloid formation in the brain. Both of these properties of M. citrifolia were experimentally observed [27-29]. Hence, there is a high likely hoo that some phytochemicals in M. citrifolia may act through inhibition of p38α MAPK. Drugs inhibiting various targets like acetycholinessterase, gamma- and beta secretases, have been tried in Alzheimer disease [45,46]. Some of the phytochemicals of M. citrifolia may act through these targets also and need further investigation. A comparative study in rabbits between M. citrifolia and memantine (a established drug for Alzheimer’s disease) on neurodegenerative disorder model suggests that M. citrifolia extract was superior to memantine in alleviating neuro-degeneration hence making it a remedy worth investigating [47]. Our screening study shows that the phytochemicals in M. citrifolia may bind to two different active sites in p38 MAPK with better stability compared to the known ligands and thereby are more likely to possess p38 MAPK inhibition property as per docking scores. Literature search revealed that extract of Aquilaria crassa which is a mixture of various phytochemicals of which balanophonin is also a component inhibited lipopolysaccharide-induced TNF-α production by attenuating p38 MAPK activation [48]. No such literature exists for isoprincepin containing plant extracts. Though literature search showed various studies which have elicited anti-inflammatory and anti-neurodegenerative actions of M. citrifolia, there are no significant studies which looked into the molecular mechanism of above actions and the phytochemicals which contribute to these properties. In our study, we have evaluated one such possible mechanism of action of phytochemicals in M. citrifolia.

CONCLUSION

Based on docking study of the 22 phytochemicals present in M. citrifolia fruit which were screened, isoprincepin and balanophonin may have p38α MAPK binding potential. Balanophonin has favorable physiochemical properties for blood-brain barrier penetration while isoprincepin may show high selectivity for p38α MAPK by bonding to Met 109 and thereby causing glycine flip phenomenon. There may be a synergistic action of these phytochemicals upon various active sites in p38α MAPK.

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REFERENCES


