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Molecular docking and interactions between vascular endothelial growth factor (VEGF) receptors and phytochemicals: An *in-silico* study

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ABSTRACT

Vascular endothelial growth factor (VEGF) is a mitogen, which plays a vital role in angiogenesis and neovascularization. Numerous medications have been developed to inhibit VEGF in cancer, because of its significance in tumor development and its survival. Hence, the current study is focused on identifying phytocompounds to directly target VEGF receptors using *in-silico* approach. In this study, a computational approach has been applied to elucidate the role of the phytocompounds in inhibition of angiogenesis through modulation of VEGF receptors responsible for angiogenesis. Molecular docking analysis was performed using AutoDock 4.2 software. In addition, the *in silico* ADMET analysis was carried out to predict the pharmacokinetic properties. Among the 50 phytocompounds tested, 6 compounds (Eriodyctiol, Epicatechin, Scutellarin, Ginkgolide A, Tetrandrine and Bassic acid) showed a better interaction profile than standard drug (Triamcinolone). Our study suggests a potential therapeutic effect of these phytocompounds for inhibiting angiogenesis, thereby providing inkling for novel drug design for proangiogenic diseases such as DR.

1. Introduction

Diabetes is a complex condition and among them, Type 1, Type 2 and gestational diabetes are affecting diverse groups of people. The effects of these nagging conditions are secondary complications like Diabetic Retinopathy (DR), neuropathy and nephropathy. Among which DR occurs commonly (Ciulla. et al., 2003). According to studies, patients with Type 2 diabetes mellitus (DM) are more prone to DR, unlike Type 1 DM. The decreased trend in Type 1 DM patients was due to consistent control of blood glucose (Khan et al., 2019). DR is the major cause of vision loss or impairment in Type 2 DM patients, and is classified into proliferative and non-proliferative DR (Heng et al., 2013). The retina experiences swollen blood vessels and leakage of fluid resulting in a condition called macular oedema. When it comes to pathogenesis, there are multiple biochemical and molecular mechanisms known to cause DR. One such molecular mechanism involves MicroRNAs (miRNA). The miRNAs are small non-coding RNA with 18–25 nucleotides capable of regulating gene expression by suppression. Vascular endothelial growth factor (VEGF) is a mitogen, which plays a vital role in angiogenesis and neovascularization. There are variants in VEGF but the maximum of the miRNAs involved in DR is up-regulated VEGF-A. Studies suggest that elevated blood glucose can cause oxidative stress characterized by an excess of reactive oxygen species (ROS), inflammation, and hypoxia etc (Singh.et al., 2008).

Angiogenesis is a complex process in which the pre-existing blood vessels give rise to the new ones. There are various chemical mediators which can regulate the angiogenic process, such as VEGF. The blood vessels grow in response to VEGF under the condition of the hypoxic retina. Increased mRNA transcription and expression of VEGF is the primary reason behind it. However, its cellular responses are stimulated by binding to tyrosine kinase receptors on the endothelial cell surface, causing them to dimerize and become activated through transphosphorylation (Temirak, 2014).

Many pathologic vascular diseases, such as macular degeneration, DR, retinal vein occlusion of the eye involves VEGF and blocking VEGF is beneficial in these diseases. Current treatments include laser therapy which ensures quite long-term efficacy, particularly for macular oedema patients. Laser therapy is considered as the golden standard of treatment, yet it is not effective in some patients (Jasmine and Vanaja, 2013). Vitrectomy is the removal of vitreous humour (Heng et al., 2013). Intra vitreal injections contain steroidal compounds to reduce capillary permeability and inflammation. Intravitreal dexam-

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ethasone, fluocinolone, and triamcinolone have been investigated so far (Jasmine et al., 2013). In the present study, Triamcinolone is used as a standard drug. Apart from these, anti-angiogenesis or anti-VEGF agents are used. Two commercially available agents are monoclonal antibodies- Ranibizumab and Bevacizumab. Recently, Retinal specialists are inclined towards anti-VEGF agents for treating this recurrent condition of angiogenesis. Phytocompounds are mainly used to treat communicable and non-communicable diseases (Ranjani et al., 2019). Hence, our study investigates a set of natural phytocompounds to target VEGF, in turn, to be used as anti-VEGF agents or as a potential drug candidate to treat DR.

2. Materials and methods

2.1. Phytocompounds

Natural bioactive constituents of plants including alkaloids, flavonoids, terpenoids and many more were selected and their 3D structure, was downloaded from PubChem in SDF format. Initially, 50 phytocompounds were selected for analysis. To compare the interactions between VEGF and phytocompounds, we had also compared with the interaction of standard drug, Triamcinolone (an intravitreal steroidal compound) to bind with VEGF. The 3D structure of the drug was also downloaded from PubChem in SDF format.

2.2. Protein preparation

The three-dimensional crystal structure of compounds was obtained from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (http://www.rcsb.org/pdb/home/home.do). in the. pdb format as 3QTK without any complexed ligands. This was processed using AutoDock 4.2 software. The protein VEGF-A was further cleaned by removing the non-essential water molecules and polar hydrogens were added and Kollman charges were computed (−18.952).

2.3. Ligand preparation

The three-dimensional structures of 50 shortlisted phytocompounds were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) in SDF format, were further converted to PDB format using Open Babel Converter (http://openbabel.org/wiki/Main_Page) to be compatible with molecular docking analysis. Torsion angles and aromaticity criteria were set for the ligand. After this, the active sites of binding in the protein were found out using online tools preferably click2drug and binding site predictor. These were used to set the grid parameters. After the minimization process, the grid box resolution was set at 60 × 60 × 60 points along the x, y, and z-axes respectively at a grid resolution of 0.375A° to define the binding site. The grid box centered in the protein is used for obtaining favorable docking conformations (Ciulla et al., 2003).

2.4. Molecular docking simulation

Following the receptor and ligand preparation, molecular docking analysis was performed by AutoDock 4.2 (http://autodock.scripps.edu/). We first docked the controls and subsequently the interactions were compared with results of shortlisted 50 phytocompounds into the active sites. The Lamarckian genetic algorithm was used to make docking calculations. Genetic Algorithm (GA) parameters were set as default. 10 runs were performed ranked according to best binding energies. The final docked results were obtained as (.dpf) file. The resulting conformations were visualized using Discover Studio Visualizer (Caldwell et al., 2003). Binding site residues were visualized along with bond length for the best binding energy conformation.

2.5. In silico analysis

2.5.1. Lipinski screening

Lipinski rule of five also called Pfizer's rule is used to evaluate the drug-likeness and durability of a phytochemical or chemical compound (Lipinski, 2004). Using this any biologically or the pharmaceutically active compound can be tested for its oral activity using this thumb rule of five. Ligands of this particular study were analyzed using http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp which analyses using five rules. The Phytocompounds satisfying this rule was subjected to docking analysis.

2.6. ADMET analysis

Any biological or pharmaceutically active compound before being selected as a drug candidate should undergo pharmacokinetic analysis (Daina et al., 2014). Bioavailability, intestinal absorption, blood-brain barrier permeability, drug-likeness, toxicity, and many other factors need to be evaluated. Ligands of our study were evaluated using the Swiss ADME online tool (http://www.swissadme.ch/). This is just to aid further investigations on formulations for our ligands/phytocompounds. Water solubility was calculated based on (ESOL Topological method implemented and lipophilicity was calculated (Diana and Zoete, 2016).

2.7. Analysis of molecular properties by molinspiration

Molinspiration is a cheminformatics software (http://www.molinspiration.com/) used to calculate important molecular properties of ligands before docking. Molecular properties include logP, polar surface area, number of hydrogen bond donors and acceptors and others, as well as prediction of bioactivity score for the most important drug targets (G-protein-coupled receptors (GPCR) ligands, kinase inhibitors, ion channel modulators, nuclear receptors) (Daina and Zoete, 2016). These properties are calculated using canonical SMILES of respective six ligands. SMILES formula was obtained from PubChem.

2.8. Molecular dynamics and simulated annealing

Docked results of six phytocompounds were subjected to dynamics and simulated annealing using SYBYL Dynamics model. Molecular dynamics and simulated annealing were performed only with ligands to observe the movement of atoms and the conformational changes of the ligand and to check its stability (Priya et al., 2018; Ubaid et al., 2018; Akther et al., 2019; Arpudhamary et al., 2019) Dynamics was carried out for 10 ns. The popular force field AMBER was used (http://ambermd.org/). Results had certain ligands with changed conformation and thereby reduced the number of bonds (Fazeela Mahaboob Begum et al., 2017a,b) This suggests that conformational changes could not support ligands to a good drug candidate and the original docking results were better (Fazeela Mahaboob Begum et al., 2017a,b).

3. Results

The molecular mechanism or the pathogenesis of DR was studied to find out the role of VEGF in neovascularization and angiogenesis in the retina. To develop a suitable drug candidate, 50 phytocompounds were screened against VEGF. In our study, 50 natural bioactive constituents of plants belonged to the class of flavonoids, terpenoids, polyphenols, lipids, and acids (Fig. 1).

All these compounds were docked with VEGF using AutoDock 4.2 software. To know if our docked ligand had interacted well, we compared it with the interaction of standard drug Triamcinolone (Fig. 2).

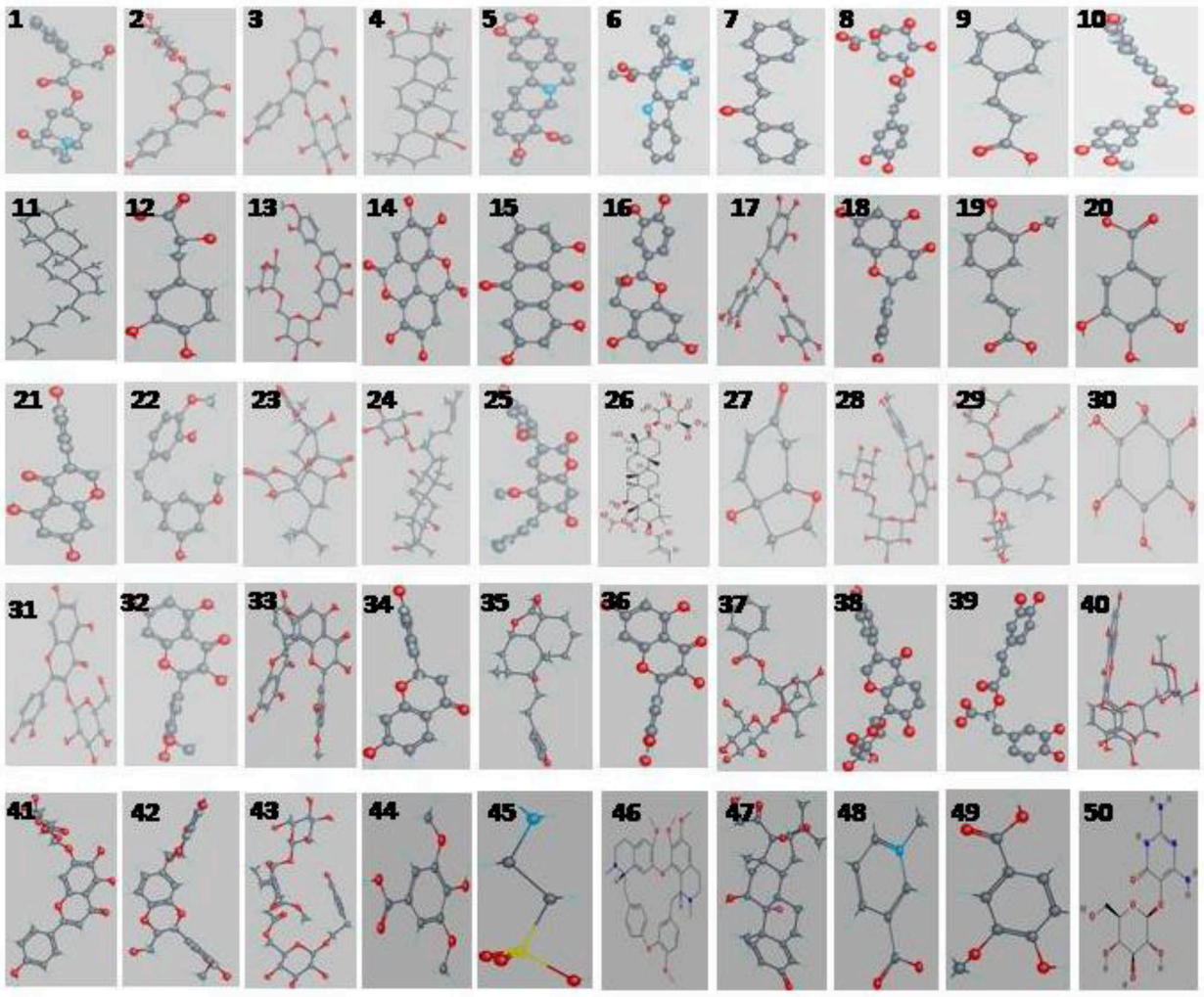

Fig. 1. Structures of 50 phytocompounds used in the study.

After docking our ligands with targeted protein, the binding energy value (kcal/mol), inhibitory constant (μm) and ligand binding site amino acids were recorded. The binding energy of our standard drug was found to be −5.18 kcal/mol with binding site amino acid to be THR70, ASP12. Among 50 ligands screened, only six phytocompounds had binding energy comparable to that of standard drug. These compounds were identified as Eriodyctiol, Epicatechin, Scutellarin, Ginkgolide A, Tetrandrine and Bassic acid and their respective binding energies are −5.42, −5.27, −5.84, −5.05, −6.02 and −5.99 kcal/mol respectively (Table 1). From the results, it is clear that these phytocompounds had relatively good binding energy and they were able to bind to VEGF just like the standard drug.

3.1. Lipinski screening

Epicatechin, Eriodyctiol and Ginkgolide A had good drug-likeness satisfying all five criteria. This proves that ligands can serve as qualified drug candidates.

Pharmacokinetic analysis performed using Swiss ADME has given important information for all the ligands which can help in further formulation and *in vitro* studies. The water solubility and lipophilicity results of the ligands were given in Table 2. All the six ligands were highly water-soluble except Bassic Acid which is moderately soluble. Among the six ligands tested Scutellarin showed the least lipophilicity (Table 2).

In order to study the dynamic results with both ligand and protein, one among the best-docked ligands (Scutellarin) that had both best binding energy and Lipinski drug-likeness was further subjected to molecular dynamic analysis using MD Simulation software with constant temperature and default parameters. Interaction results of docking, dynamics and simulated annealing were compared and the stability of the interaction was confirmed. With this simulated analysis, the simulated structure was not affected critically and RMSD didn't cross 2 Å away from the initial structure. The Radius of gyration also remained at 20 Å (Fig. 3) (Priya et al., 2018) Fig. 4 Scutellarin conformation before and after simulated annealing showed disappearance of bonds (Fig. 4).

Further, the drug ability of ligands was studied by calculating properties such as kinase inhibitors (KI), protease inhibitors (PI), ion channel modulators (ICM), nuclear receptor ligands (NRL), enzyme inhibitors (EI) and ligands as GPCR. All these properties were calculated as bioactivity scores. Scores of >0.00 represent high activity, while scores between 0.00 to −0.5 represent moderate activity and scores <-0.5 represent inactivity. Among all the legands Tetrandrine satisfied all the criteria to be used as potential lead compound when it was compared with other ligands (Table 3) Based on NRL, all the compounds except tetrandrine showed good activity scores with potential as promising therapeutic agents (Table 4). The larger the value of the score, the higher will be the probability that the particular molecule will be active.

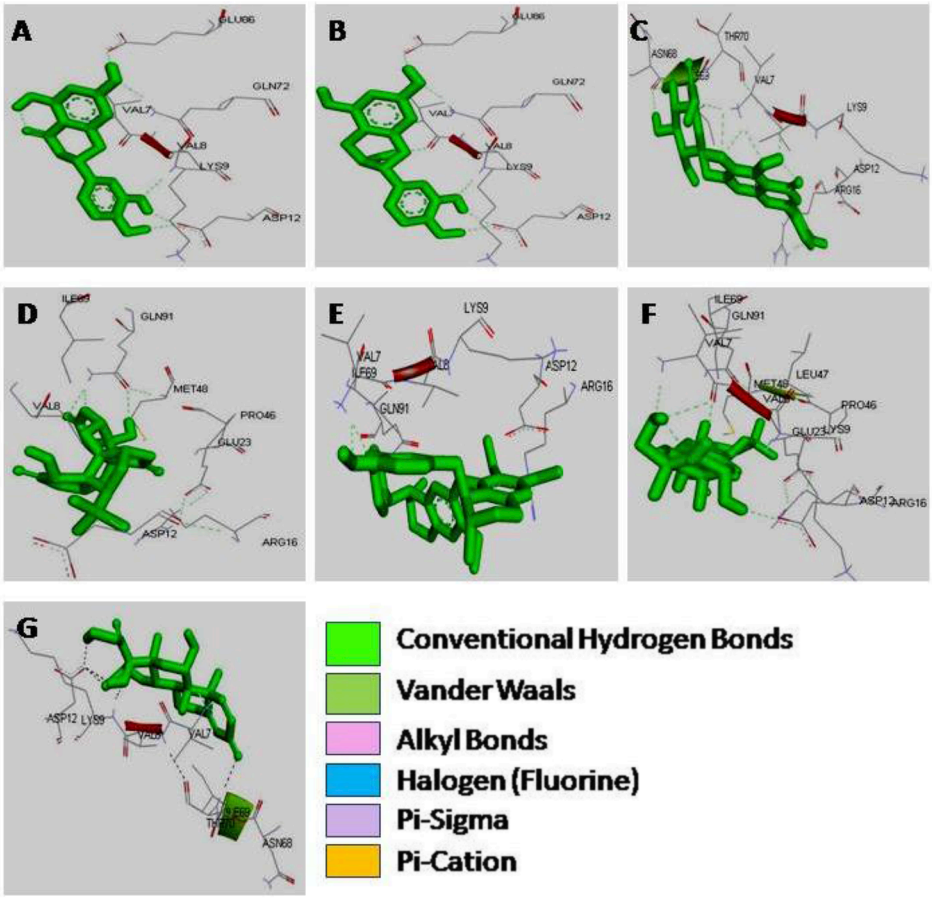

Fig. 2. Interaction of VEGF with (A) Eriodyctiol (B) Epicatechin (C) Scutellarin (D) Ginkgolide A (E) Tetrandrine (F) Bassic Acid and (G) standard drug Triamcinolone using Discover studio visualizer.

Table 1
Interaction results of best-suited compounds compared to standard drug.

S.No	Phytocompounds	Binding Energy (Kcal/Mol)	Ligand Binding Site Residue	No. of Hydrogen Bonds	Vanderwaal's Residue
Drug	Triamcinolone	−5.18	THR70, ASP12	2	LYS9, ASN68
1	Eriodyctiol	−5.42	GLN72, ASP12, GLU68	3	LYS9, ASN68
2	Epicatechin	−5.27	VAL7, LYS9, GLN72, GLU86, ASP12	5	VAL8
3	Scutellarin	−5.84	VAL7, ARG16, THR70, ASN68	4	LYS9, ILE69
4	Ginkgolide A	−5.05	GLN91	1	ASP12, GLU23
5	Tetrandrine	−6.02	VAL7, ARG16	2	LYS9, ASP12, ILE69
6	Bassic acid	−5.99	VAL7, ASP12	2	LYS9, ARG16, GLU23, GLN91, LEU47

Table 2
Swiss ADMET analysis.

Ligands	Water Solubility	Gi Absorption	Bbb Permeant	P-Gp Substrate	Lipophilicity
Eriodyctiol	−3.26 (Soluble)	High	No	Yes	1.62
Epicatechin	−2.22 (Soluble)	High	No	Yes	1.47
Scutellarin	−3.27 (Soluble)	Low	No	Yes	1.11
Ginkgolide A	−2.68 (Soluble)	High	No	Yes	1.56
Bassic Acid	−5.91 (Moderately soluble)	High	No	Yes	3.32
Tetrandrine	−8.02 (Poorly soluble)	High	No	No	5.16

4. Discussion

Angiogenesis is a complex physiological process through which the pre-existing blood vessels give rise to new ones. It involves the migration, growth, and differentiation of endothelial cells within the wall of blood vessels (Carmeliet and Jain, 2000). Angiogenesis plays an important role in tumor growth and metastasis (Nishida et al., 2006). Angiogenesis is controlled by chemical signals in the human body. Among the chemical signals, VEGF plays a vital role in angiogenesis. VEGF is expressed at the very early stages of growth in most of the tumors (Ferrara et al., 2003). As a part of cancer treatment modalities, anti-VEGF medication is currently used in which, the mechanism of tumor resistance to VEGF medication is the main target.

In this study, *in silico* molecular docking and interaction studies were performed to explore the therapeutic potentials of various phytocompounds to inhibit angiogenesis through modulation of VEGF receptors. Upon comparison with the standard drug (triamcinolone) which is widely used intravitreal corticosteroid injection, 6 phytocompounds showed the potential to be used as a drug candidate. All 6 compounds interacted well with VEGF. Based on binding energy, Tetrandrine had the best binding energy score of – 6.02 kcal/mol with 2 ligand binding site residues (VAL and ARG). Binding energy gives the strength of interaction and affinity of the compound to bind with a particularly active site of the target protein. Lower the binding energy better is the interaction. However, based on hydrogen bonds or

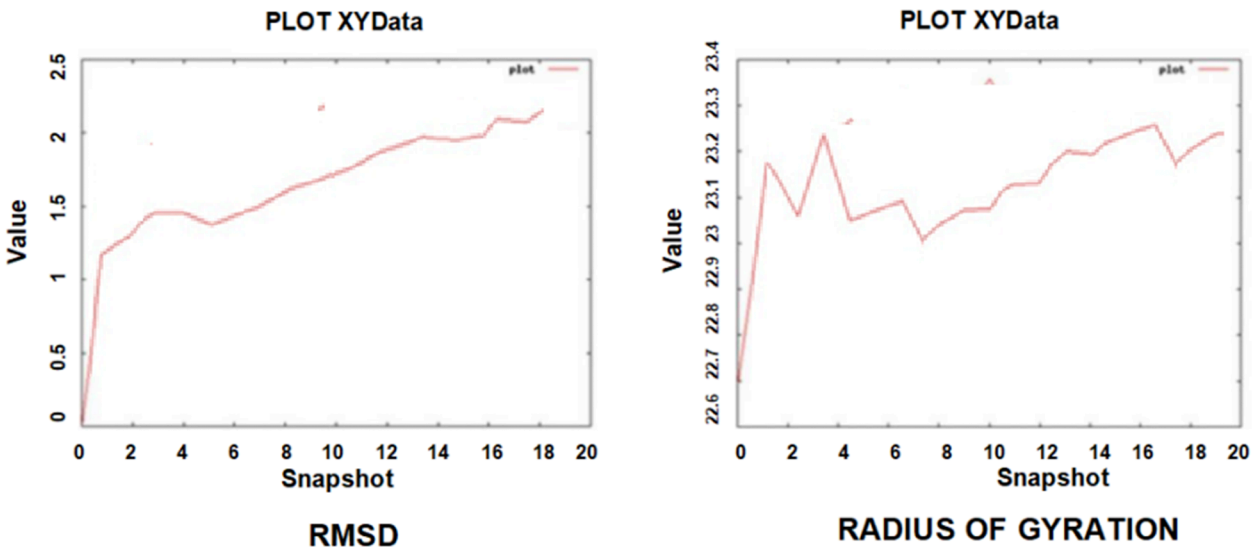

Fig. 3. Dynamic results of scutellarin using MD Simulation software.

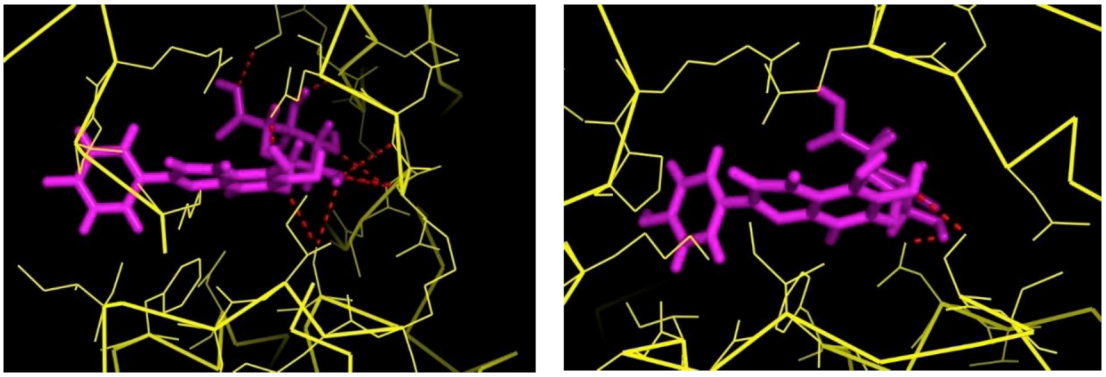

Fig. 4. Scutellarin conformation before and after simulated annealing showing disappearance of bonds. Table 3

Table 3
Physicochemical parameters of the ligand.

Ligand	GPCR	ICM	KI	NRL	PI	EI
Eriodyctiol	0.07	−0.20	−0.22	0.46	−0.09	0.21
Epicatechin	0.41	0.14	0.09	0.60	0.26	0.47
Scutellarin	0.08	−0.09	0.01	0.33	−0.05	0.41
Ginkgolide A	1.15	0.07	−0.29	0.14	0.34	0.35
Tetrandrine	−0.11	−0.77	−0.62	−0.71	−0.13	−0.44
Bassic Acid	0.23	−0.28	−0.35	0.79	0.16	0.66

GPCR: G-protein-coupled receptors; ICM: Ion channel modulators; KI: Kinase inhibitors; NRL: Nuclear receptor ligands; PI: Protease inhibitors; EI: Enzyme inhibitors.

Table 4
Estimation of drug ability of the ligand.

Ligand	miLogP	TPSA	MW	Nrotb
Eriodyctiol	1.63	107.22	288.25	1
Epicatechin	1.37	110.37	290.27	1
Scutellarin	0.07	207.35	462.36	4
Ginkgolide A	−1.46	128.60	408.40	1
Tetrandrine	6.55	61.88	622.76	4
Bassic Acid	4.45	97.98	486.69	2

the number of ligand binding sites, Scutellarin had the best results with 4 hydrogen bonds (VAL, ARG, ASN and THR) and a binding energy score of −5.84 kcal/mol. Nuclear receptors (NRL) are major pharmaceutical targets since they are known to play an important role in diabetes and its regulation (Gupta et al., 2013). Further *in-silico* analysis of these compounds predicted drug-likeness and ADMET properties. Lipinski drug-likeness with input pH value 7.0 revealed eriodyctiol, epicatechin and ginkgolide A to satisfy all the criteria suited for a drug candidate. Similarly, ADMET analysis was also done so that it can aid future investigators during drug formulations. Molinspiration gives the physiochemical properties of the ligand. Before and after docking, these parameters aid different aspects of drug development.

There are various techniques currently used to block the VEGF pathway in which, one of the important one is neutralizing monoclonal antibodies against VEGF or its receptor (Cardones and Banez, 2006). Research suggests that anti- VEGF molecules alone can block the angiogenesis and delay tumor progression (Carmeliet and Jain., 2000). However, the mechanisms involving the destruction of tumor blood vessels by anti-VEGF therapy and investigations of anti-VEGF therapies as potential anti-cancer treatment modalities are under exploration (Ellis and Hicklin, 2008). Similarly, the use of anti-VEGF therapies has been widely used in various diseases such as ocular diseases, including Neovascular Age-related Macular Degeneration (NVAMD), DR, and retinal vein occlusions (Kim and D'Amore PA., 2012). Moreover, VEGF is a vital factor in ocular homeostasis (Tolentino, 2011). Recent strategies to reduce the risk of DR are good

management of blood glucose and blood sugar (Parveen et al., 2018). Upon incidence, the therapeutic strategies range from laser photocoagulation to vitrectomy. Although all these invasive and surgical procedures are quite successful, they have side effects and risk of permanent damage to the retinas. This led to a new beginning of using anti-angiogenesis agents to directly inhibit angiogenesis. Initially, certain agents that were found effective in controlling tumor growth have been experimented for their efficacy in retardation of neovascularization. Interferon-alpha-2a, thalidomide are few drugs that were in clinical trials and were quite successful in targeting VEGF (Ciulla et al., 2003). With all the current surgical treatments having side effects of recurrence or permanent vision loss, plant compounds play a significant role wherein the degree of permanent damage is far reduced. Since these compounds are derived from natural sources, their availability, cost and formulation become easy (Tarr et al., 2013).

These bioactive constituents are known to be involved in the anti-angiogenesis process. Therefore, further investigations on its pharmacokinetic and dynamic properties is required to be formulated as a successful drug candidate. In addition, these compounds can also be investigated for their pharmacodynamics and kinetic properties using *in vitro* and *in vivo* approaches.

5. Conclusion

In this study, *in silico* analysis was performed to explore the therapeutic potential of various phytocompounds to inhibit angiogenesis through modulation of VEGF receptors. The overall analysis suggested that the phytocompounds have the potential to firmly bind with VEGF and retard its development. These compounds can be further investigated using *in vitro* and *in vivo* studies for its pharmacodynamics and kinetic properties.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101424.

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