ORIGINAL ARTICLE

In silico studies on phytoconstituents of *Vernonia arborea* Buch. - Ham. against Mitogen-activated protein kinase-I

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Abstract

Introduction: The present study focuses on *in silico* docking of phytocompounds eugenol and vanillin from the plant *Vernonia arborea* against mitogen-activated protein kinase-I (MAPK-I) (PDB ID – 4U7Z) to assess their anti-inflammatory potential. **Materials and Methods:** Gas chromatography-mass spectrometry analysis was carried out on the ethanolic leaf extract of *V. arborea* and the phytoconstituents present was identified after comparison with the NIST library. *In silico* analysis of the selected compounds was done by AutoDock software. Indomethacin was used as the reference compound for the study. **Results:** The results showed that eugenol interacted with the amino acid residues MET106 and ASP104 in the active site of MAPK-I with a bond length of 1.864 Å and 1.8 Å, respectively, and the IC_{50} value of this compound was found to be 3.21 (µm). The second ligand, vanillin showed interactions with the amino acid residues MET106, THR 108, and LYS112 with a bond length of 1.991 Å, 2.243 Å, and 2.166 Å, and the IC_{50} value was 3.29 (µm). Indomethacin showed interactions with methionine and lysine at a bond length of 2.165 Å and 2.007 Å with an IC_{50} value of 9.21 (µm). **Discussion and Conclusion:** The ligands formed an effective hydrogen bonding with the chosen target and the bonds formed were well within the range of >1.5 Å and <3.2 Å. The median inhibitory concentration values of the selected phytocompounds were also a proof of their superior anti-inflammatory potential when compared to indomethacin. Based on these promising preliminary *in silico* results, further *in vitro* and *in vivo* experiments can be planned to evaluate the anti-inflammatory activity of *V. arborea*.

Key words: Eugenol, indomethacin, mitogen-activated protein kinase-I, vanillin, *Vernonia arborea*

INTRODUCTION

The cellular inflammatory process is a complex interplay between cells of the blood, blood vessels, and cells of the injured tissue and is a coordinated response of cells to an initial stimulus.[1] These inflammatory responses are required for immune surveillance, optimal repair, and regeneration after injury.[2] However, inflammations if runs unchecked may lead to a number of diseases such as arthritis, atherosclerosis, insulin resistance, and cancer. Medications that work to reduce inflammation come in two major categories: steroids (e.g., cortisone) and non-steroidal anti-inflammatory drugs (NSAIDs). Long-term use of corticosteroids results in various side effects such as hyperglycemia, insulin resistance, diabetes mellitus, osteoporosis, and anxiety effects.[3] NSAIDs, when used continuously, may cause a 4–7-fold increased risk of gastric injury.[4]

Phytomedicinal research has a good chance of contributing new strategies through the development of new and better drugs for an evidence-based and rational phytotherapy. Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques.[5] The medicinal flora of Asia and Pacific comprises a fantastic source of pharmacologically active products, and the number of plant species principally used for the treatment of inflammation can be estimated to be more than 380.

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Different species of *Vernonia* have been employed in traditional medicine for long and have been used in treatment for many pathological conditions. The genus *Vernonia* produces characteristic compounds such as sesquiterpene lactones,[6] flavonoids,[7] steroids,[8] and polysaccharides.[9] The bark juice of *V. arborea*, a medium-sized trees species, has been used to treat worms, infusion of roots, or decoction is given in fever. In southern Sumatra, the bark is chewed at the first sign on sprue. The aqueous and methanolic extracts of *V. arborea* were found to possess wound healing activity[10] and the plant has also been reported to possess hepatoprotective activity against carbon tetrachloride induced hepatic damage in rats.[11]

The aim of the present study is to evaluate the *in silico* docking effect of two compounds eugenol and vanillin identified from *V. arborea* through gas chromatography-mass spectrometry (GC-MS) analysis against the target mitogen-activated protein kinase-I (MAPK-I).

MATERIALS AND METHODS

Plant Material

The leaves of the plant *V. arborea* were collected from Kolli Hills, Eastern Ghats, Tamil Nadu, India. Botanical identification of the plant was done by Prof. Dr. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy Research Center, Tambaram, Chennai, Tamil Nadu, India, and a voucher specimen was deposited (Voucher No. PARC/2012/1239) at Rapinat Herbarium (Voucher No. SJCBOT 1202), Department of Botany, St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India.

GC-MS Analysis

The GC-MS was performed using Perkin Elmer Clarus 500 equipped with a column type capillary column Elite-5 (5% phenyl and 95% dimethylpolysiloxane), with a column length of 30 m/0.25 mm and a film thickness of 250 µm, respectively. The residue was diluted (1 µl/ml) and 1 µl was taken as the injection volume. They were injected in the split mode with 10:1 ratio. The oven was programmed at 50°C at 8°C/min to 220°C (2 min) at 8°C/min to 290°C (10 min.) and the injector was maintained at 290°C. Helium was used as a carrier gas with a constant flow at 1 ml/min. The ionization voltage was 70 eV. The constituents were identified after comparison with those available in the computer library (NIST) attached to the GC-MS instrument and the results obtained are reported.[12]

Selection of Ligands

The compounds identified through GC-MS analysis were subjected to a thorough literature review, and those compounds with proven anti-inflammatory activity were chosen as ligands for the *in silico* study.

Selections of Target Molecule

The structure of the selected target (PDB ID – 4U7Z) was retrieved from the PDB database and molecular docking studies were carried out by targeting its active amino acid residues. Indomethacin was used as the reference compound.

Active Site Prediction

The active site of the protein is its binding site or usually a pocket at the surface of the protein that contains residues responsible for substrate specificity which often act as proton donors or acceptors. Identification and characterization of the binding site are the key step in structure-based drug design. The binding site was identified by computational and literature reports. The active site region of the protein was identified by Q–site.[13] These servers analytically furnished the area and the volume at the probable active site of each pocket to envisage the binding site.

Docking Protocol

Grid parameter files (protein.gpf) and docking parameter files (ligand.dpf) were written using MGLTools-1.4.6. Receptor grids were generated using 90 × 60 × 60 grid points in xyz with grid spacing of 0.375 Å. Grid box was centered, and co-crystallized ligand map types were generated using autogrid 4. Docking of macromolecule was performed using an empirical free energy function and Lamarckian genetic algorithm, with an initial population of 250 randomly placed individuals, a maximum number of 106 energy evaluations, a mutation rate of 0.02, and a crossover rate of 0.80. One hundred independent docking runs were performed for each ligand. Results differing by 2.0 Å in positional root-mean-square deviation were clustered together and represented by the result with the most favorable free energy of binding.

Absorption, Distribution, Metabolism, Excretion (ADME) Toxicity Prediction

Plant compound inhibitor can also be optimized by computational ADME prediction as it is crucial in inhibitor designing. In the present investigation, the ADME properties of the selected compounds were studied using a support vector machine classification algorithm[14] to take it further for inhibitor designing.

RESULTS AND DISCUSSION

The results of GC-MS analysis of ethanolic extract of *V. arborea* showed the presence of 63 compounds. Among these, based

on detailed literature study, eugenol and vanillin were selected for *in silico* studies. The two chemical constituents along with their molecular formula, molecular weight, retention time, and percentage area are given in Table 1.

Protein Preparation

The structure of the selected target MAPK-I was retrieved from the PDB database (PDB ID – 4U7Z). The protein MAPK-I [Figure 1] was assigned with Kollman charges that aided in addition of hydrogens, and side chains were optimized for hydrogen bonding. The energy minimized protein was then saved in PDB format. Using MGLTools-1.4.6, the nonpolar hydrogens were merged, AutoDock atom type AD4 and Gasteiger charges were assigned and finally saved in protein.pdbqtformat.[15]

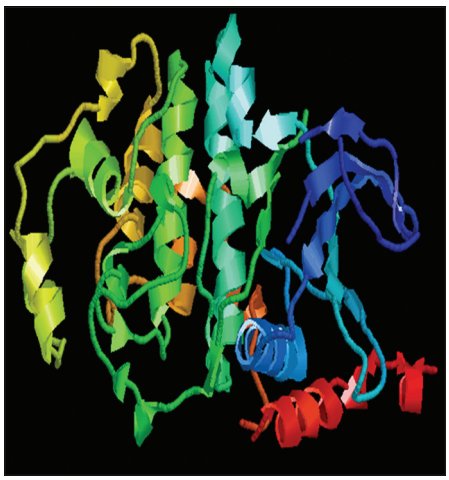

Figure 1: Rasmol view of mitogen-activated protein kinase I

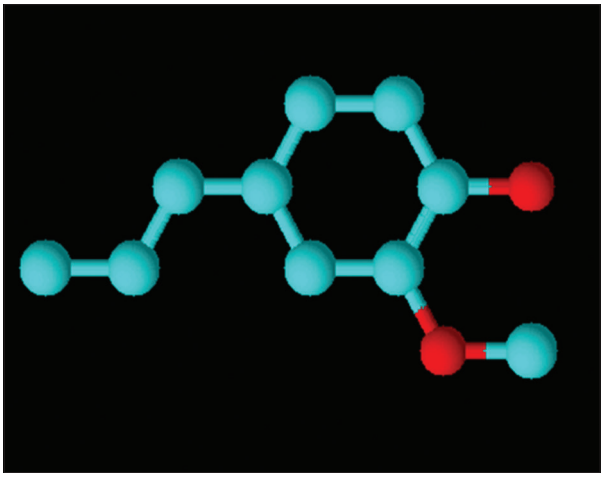

Figure 2: Eugenol

Ligand Preparation

Structure of ligands was drawn using ChemSketch, optimized with 3D geometry and the two-dimensional structure of eugenol, vanillin, and indomethacin was converted into 3D structure [Figures 2-4] using the open Babel format molecule converter[16] and saved in PDB format for AutoDock compatibility. MGLTools-1.4.6 (The Scripps Research Institute) was used to convert ligand.pdb files to ligand.pdbqt files.

ADME Toxicity Prediction

Inadequate ADME properties are the cause of many drug development failures.[14] In the present study, a proper ADME toxicity prediction of the selected compounds was done and the results obtained possessed all the characteristics of the plant molecule such as molecular weight, computed dipole

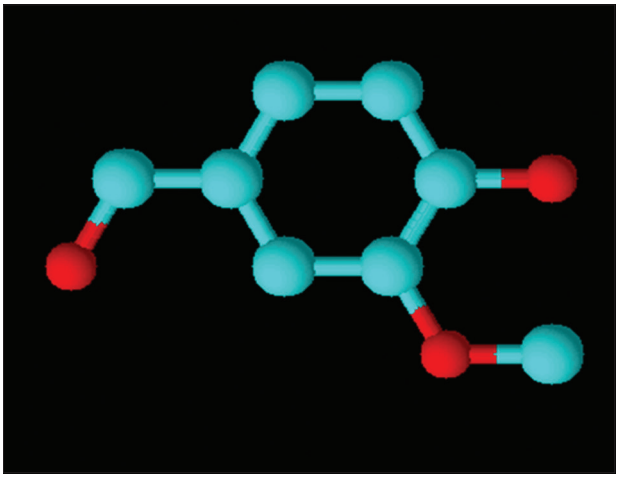

Figure 3: Vanillin

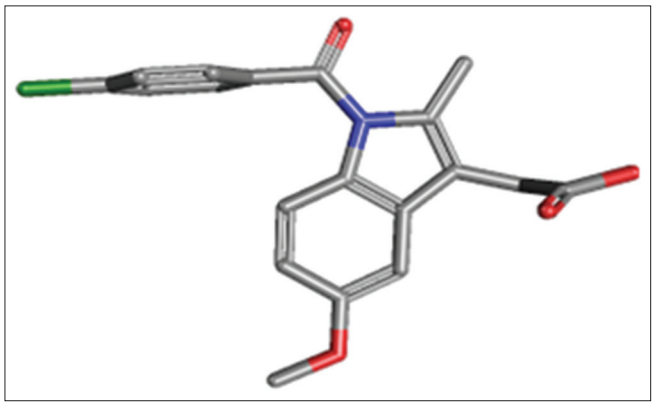

Figure 4: Indomethacin

Table 1: GC-MS phytocompounds selected for *in silico* study

Peak name	Retention time	Peak area	% Peak area
Name: Eugenol Formula: $C_{10}H_{12}O_2$ MW: 164	14.93	242274288	22.8249
Name: Vanillin Formula: $C_8H_8O_3$ MW: 152	15.86	490052	0.0462

GC-MS: Gas chromatography-mass spectrometry

moment of the molecule. Ionization potential, electron affinity, π (carbon and attached hydrogen) component of the solvent accessible surface area, weakly polar component of the solvent accessible surface area, polar surface area, volume, #rotor, donor hydrogen bond (HB), and accept HB which were very essential to determine if the selected ligand possessed the desired biological activity [Table 2].

Docking Studies

AutoDock is an automated docking tool designed to predict the binding of small molecules, such as substrates or drug candidates, to a receptor of known 3D structure.[17] Amino acid residues in the domain region may have a central role in its functions and are also believed to forge a structure-function relationship of a protein in its tertiary conformation. In the present study, the active site of the target MAPK-I comprised the amino acid residues methionine (MET106), aspartate (ASP104), lysine (LYS112), isoleucine (ILE29), and leucine (LEU154). Docking analysis of the selected ligands and the target is given in Table 3.

The results showed that the first ligand, eugenol interacted with the amino acid residues MET106 and ASP104 in the active site of MAPK-I with a bond length of 1.864 Å and 1.8Å, respectively, and the IC_{50} value of this compound was found to be 3.21 (µm) [Figure 5].

The second ligand, vanillin showed interactions with the amino acid residues MET106, THR 108, and LYS112 with a bond length of 1.991 Å, 2.243 Å, and 2.166 Å, and the IC_{50} value of this compound was found to be 3.29 (µm) [Figure 6].

The reference drug indomethacin showed two interactions with the amino acid residues MET106 and LYS112 [Figure 7].

The length of HB formed was 2.165 Å and 2.007Å with an IC_{50} value of 9.21 (µm). The hydrogen bonding established between the chosen phytocompounds and MAPK-I was within the acceptable range of >1.5 Å and <3.2 Å, and the IC_{50} values of the ligands chosen were lesser than the

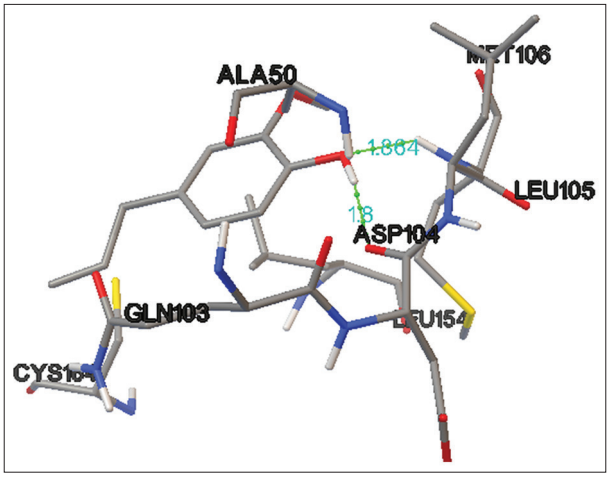

Figure 5: Docking of Eugenol with mitogen-activated protein kinase-I (MAPK-I)
Pictorial representation of docking of Eugenol with MAPK-I (Note: MET106 N-H…O; Bond length 1.864; ASP104 O-H…O; Bond length 1.8; AutoDock score: −9.75 K Cal/Mol; IC_{50} value − 3.21).
Cluster rank = 1
Estimated free energy of binding = −5.13 kcal/mol
Final docked energy = −9.75 kcal/mol
1. IC_{50} value = 3.21
2. H-bond interaction = MET106 N-H…O
3. Bond distance = 1.864 Å
Final docked energy = −9.75 kcal/mol
1. IC_{50} value = 3.21
2. H-bond interaction = ASP104 O-H…O
3. Bond distance = 1.8

Table 2: ADME toxicity prediction for the selected plant molecules

Compound name	Mol.Wt.	log P	tPSA	Rotatable bonds	Rigid bonds	HB donors	HB acceptors
Eugenol	164.20	2.61	29.46	3	7	1	2
Vanillin	152.15	1.00	46.53	2	7	1	3
Indomethacin	357.79	4.00	68.53	4	19	1	5

ADME: Absorption, distribution, metabolism, excretion

Table 3: Molecular docking studies of plants compounds with MAPK-I

Compound name	Docking score	IC_{50} value	H-Bond interaction	Distance
Eugenol	−9.75	3.21	MET106 N-H…O ASP104 O-H…O	1.864 1.8
Vanillin	−7.48	3.29	MET1O6 N-H…O THR 108 O-H…O LYS112 N-H….O	1.991 2.243 2. 166
Indomethacin	−5.78	9.21	MET1O6 N-H…O LYS112 N-H…O	2.165 2.007

MAPK-I: Mitogen-activated protein kinase-I

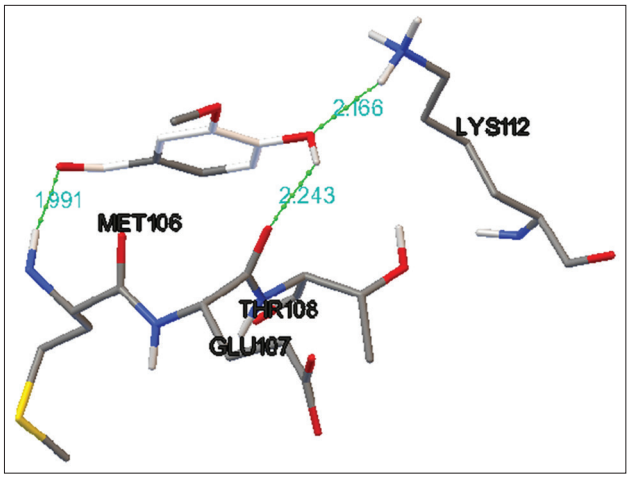

Figure 6: Docking of vanillin with mitogen-activated protein kinase-I (MAPK-I).
Pictorial representation of docking of vanillin with MAPK-I (Note: MET106 N-H…O; Bond length 1.991; THR 108 O-H…O; Bond length 2.243; LYS112 N-H….O; Bond length 2. 166; AutoDock score: −7.48 K Cal/Mol; IC_{50} value – 3.29).
Cluster rank = 1
Estimated free energy of binding = −4.62 kcal/mol
Final docked energy = −7.48 kcal/mol
1. IC_{50} value = 3.29
2. H-bond interaction = MET106 N-H…O
3. Bond distance = 1.991 Å

Final docked energy = −7.48 kcal/mol
1. IC_{50} value = 3.29
2. H-bond interaction = THR 108 O-H…O
3. Bond distance = 2.243

Final docked energy = −7.48 kcal/mol
1. IC_{50} value = 3.29
2. H-bond interaction = LYS112 N-H….O
3. Bond distance = 2.166

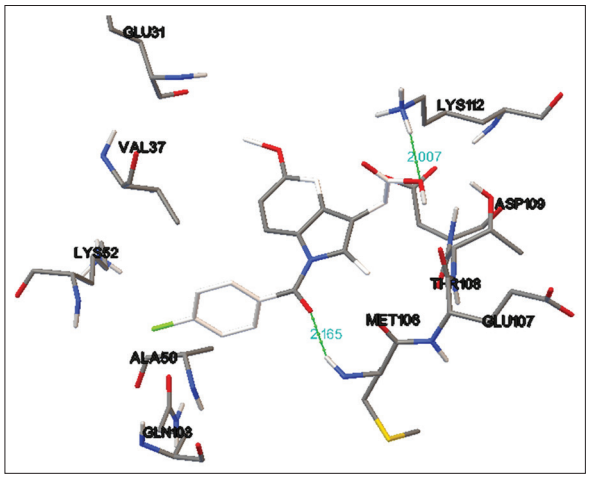

Figure 7: Docking of Indomethacin with mitogen-activated protein kinase-I (MAPK-I)
Pictorial representation of docking of indomethacin with MAPK-I (Note: MET106 N-H…O; Bond Length 2.165; LYS112 N-H…O; Bond Length 2.007; AutoDock score: −5.78 Cal/Mol; IC50 value – 9.21).
Cluster rank = 1
Estimated free energy of binding = −6.67 kcal/mol
Final docked energy = −5.78 kcal/mol
1. IC_{50} value = 9.21
2. H-bond interaction = MET106 N-H…O
3. Bond distance = 2.165 Å

Final docked energy = −5.78 kcal/mol
1. IC_{50} value = 9.21
2. H-bond interaction = LYS112 N-H…O
3. Bond distance = 2.007

reference drug which is an important criteria for an effective anti-inflammatory activity.

CONCLUSION

In the present study, two compounds eugenol and vanillin were identified from the plant *V. arborea* through GC-MS analysis and were successfully docked with the target MAPK-I. The results of docking score and IC_{50} values were indicative of a more effective docking potential of the selected phytocompounds with the target than indomethacin, the reference compound.

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