Inhibition of Aldose Activity by Essential Phytochemicals of Cymbopogon Citratus (DC.) Stapf

Vyshali P. *vyshalipingle@gmail.com*
Department of Microbiology and Biotechnology,
Jnana Bharathi campus,
Bangalore University,
Bangalore-560056, India

K.J. Thara Saraswathi *dr.tharabiotech@gmail.com*
Department of Microbiology and Biotechnology,
Jnana Bharathi campus,
Bangalore University,
Bangalore-560056, India

Rajeshwari D Sanakal *rajkbio@rediffmail.com*
Bioinformatics Infrastructure Facility Centre
Post-Graduate Department of Biotechnology and Microbiology
Karnatak University,Dharwad- 580003
Karnataka, India

B. B. Kaliwal *b_kaliwal@yahoo.com*
Professor,Bioinformatics Infrastructure Facility Centre,
Post-Graduate Department of Biotechnology and Microbiology
Karnatak University,Dharwad – 580003
Karnataka, India

Abstract

The ambiguity of whether aldose reductase, an enzyme of polyol pathway, is linked to diabetes and its complication has been receded based on the recent studies made on the inhibition of its (Aldose reductase) activity. In our current study, we have used an *in silico* approach (molecular docking) to analyze the effect of essential phytochemicals obtained from *Cymbopogon citratus* on the aldose reductase activity. *C.citratus* is grown extensively in tropical countries including India for perfumery and pharmaceuticals. The essential phytochemicals of *C.citratus* like Myrcene, Citral, and Geraniol have been used as ligand for the molecular docking analysis with Aldose reductase as receptor. The docking analysis showed Myrcene, with binding energy of -8.76 Kcal/mol is best amongst Citral and Geraniol which are having binding energies of -7.24 Kcal/mol and -7.93 Kcal/mol respectively for inhibiting the activity of Aldose reductase.

Keywords: Aldose Reductase, Molecular Docking, Citral, Geraniol, Myrcene, Cymbopogon citratus

1. PRELIMINARIES

1. INTRODUCTION

From the past few decades the question of whether polyol pathway is responsible for diabetes retinopathy [2, 3, and 4] and other complications of human diabetes have been raised many times. Substantial amount of research on polyol pathway has revealed that the inhibition of aldose reductase activity [5] can lead to the cure of diabetic retinopathy. In this paper, we have presented an *in silico* analysis on the inhibition of aldose activity by the essential phytochemicals of *Cymbopogon citratus* [6]. Various species of *Cymbopogon* have been reported to control the hyperglycemia [7, 8] and other complication of diabetes. The essential phytochemicals were

extracted as stated in [1]. For the *in silico* analysis we have used Auto Dock [9] for the binding of ligand (essential phytochemicals of *C.citratus*) and receptor (Aldose reductase).

The hazardous polyol pathway of glucose metabolism becomes active when intracellular glucose levels are elevated [10, 14]. Aldose reductase (AR), the first and rate-limiting enzyme in this pathway, reduces glucose to sorbitol using NADPH as a co-factor; sorbitol is then metabolized to fructose by sorbitol dehydrogenase that uses NAD+ as a cofactor. The polyol pathway leads to several damaging effects. Sorbitol being an alcohol, is polyhydroxylated, and strongly hydrophilic, does not diffuse readily through cell membranes and accumulates intracellularly with possible osmotic consequences [10]. The fructose produced by the polyol pathway can become phosphorylated to fructose-3-phosphate [15, 16], which is broken down to 3-deoxyglucosone; both compounds are powerful glycosylating agents that enter in the formation of advanced glycation end products (AGEs) [15]. The usage of NADPH by AR may result in fewer co-factors available for glutathione reductase, which is critical for the maintenance of the intracellular pool of reduced glutathione (GSH). This would lessen the capability of cells to respond to oxidative stress [17]. Compensatory increased activity of the glucose monophosphate shunt, the principal supplier of cellular NADPH, may occur [17]. The usage of NAD by sorbitol dehydrogenase leads to an increased ratio of NADH/NAD+, which has been termed as "pseudohypoxia" and linked to a multitude of metabolic and signaling changes known to alter cell function [18]. It has been proposed that the excess NADH may become a substrate for NADH oxidase, and this would be a mechanism for generation of intracellular oxidant species [19]. Thus, activation of the polyol pathway, by altering intracellular tonicity, generating AGEs precursors, and exposing cells to oxidative stress perhaps through decreased antioxidant defenses and generation of oxidant species, can initiate and multiply several mechanisms of cellular damage.

Retinal ganglion cells, Muller glia, and vascular pericytes and endothelial cells are endowed with aldose reductase in all species studied, including humans [20]. Hence, these cell types are exposed to polyol pathway activation in diabetes. These are also the cells that manifest the best-known changes or damage in diabetes [21]. The biochemical consequences of polyol pathway activation have been studied in the whole retina of diabetic animals. The best-documented are the accumulation of sorbitol and fructose [22, 23], and the generation or enhancement of oxidative stress. Insofar as indices of polyol pathway-induced oxidative stress are measurable in preparations of the whole retina, the abnormalities are likely to occur in most cell types or at least in cells that are highly represented in the whole retina. Muller glia cells are the candidates because they are large cells present in high number in the retina [24].

1.1. Cymbopogon Citratus

Cymbopogon citratus (DC.) Stapf. (West Indian lemongrass or citron grass), *C.flexuosus* (steud) Wats. (East Indian lemongrass or Cochin lemongrass) and *C.pendulus* (nees) Wats (North Indian lemongrass) [1, 6]. They are extensively cultivated besides their wild status in South and Central America and many other tropical countries including India for perfumery and pharmaceuticals. *C.citratus* is well known to produce essential oils up to 1.5 %(dry weight) with characteristic 'lemon aroma' dominated by Citral, which is a mixture of isomeric, acyclic, monoterpene aldehydes (Guenther, 1950; Weiss, 1997). According to the reports, lemon grass oil consists of 75-85% Citral, which is natural geranial (Trans – Citral, Citral -A) and 40% neral (Citral, Citral-B), Geraniol (aromatic alcohol), and Myrcene (characteristic in C.citratus) (Formacek and Kubeezka, 1982). Citral is used as a raw material largely in perfumery, cosmetics and pharmaceutical industries.

Earlier studies made by the author on *C.citratus* [1] was focused on the generation of *in vitro* variants (long and short morphotypes) using young leaf segments. Further, the essential oils were extracted; GC analysis were made on long and short morphotypes and compared with controlled plants.

1.2. Auto Dock

The field of molecular docking and computer- assisted drug design from the last few decades has started to be a most important field. Now one can find a few programs that work with various accuracy and features, e.g. Auto Dock [9], DOCK [25], and Sybyl's [26]. A common feature in these programs is that they simulate or match, and in this way try to optimize the binding conformation between the two molecules. They use different techniques to accomplish this, e.g. simulated annealing [27], genetic algorithms [28], flexible molecular bonds, rigid bodies, spheres and grid boxes. In the present work we have used Auto Dock exclusively along with one of its search method called Lamarckian genetic algorithm (LGA) [29]. The macromolecule is rigid and fixed while the ligand is flexible and can both translate and rotate. Auto Dock uses a rapid grid-based method for finding the lowest binding energy of the bound conformation. These grids are calculated in advanced (i.e. before the actual docking) and one for each atom type present. The size of the grid box can be set manually and placed at a certain position. These boxes create maps over the molecules that are used during the docking, to exclude atoms of no interest and also to speed up the docking calculations.

2. PRELIMINARIES

2.1 Methodology

For the molecular interaction between the essential phytochemicals obtained from *C. citratus* [1] and the enzyme Aldose Reductase [30], we have used Auto Dock. Although, Autodock uses a wide range of algorithm like simulation annealing , genetic algorithm , Lamarckian algorithm etc, but, for the biomolecular interaction analysis between the receptor (Aldose) and ligand (phytochemicals obtained from *C. Citratus*) we have used Autodock's Hybrid Lamarckian Genetic Algorithm LGA [29]. Hybrid Lamarckian Genetic Algorithm utilizes Lamarckian notation that is an adaptation of an individual to its environment which can be inherited by its offspring. Prior to the docking analysis by using autodock, the ligand and the receptor molecule should be modeled in such a way that they contain the entire parameter and set of values, which are required by LGA while docking. The autodock experiment usually divides the ligand and the receptor into five different input files.

- A pdbqt [31] file for ligand that encodes the torsion tree
- A pdbqt file for receptor
- A Grid Parameter File(GPF) for the autogrid calculation
- A Docking Parameter File (DPF) for autodock calculation
- A pdbqt file containing the flexible residues

Both receptor and the ligand can be modeled into the above mentioned files in the following way

2.1.1 Preparing the Receptor file

Aldose reductase which we have taken as our receptor molecule for the docking analysis with the essential phytochemicals of *C.citratus* was obtained from the Brook haven protein data bank [32] with resolution of 1.65A°. The PDB file [1ADS] was first edited by adding all the hydrogen bonds to it. The catalytic site of 1ADS consists of residues such as Asp, Tyr, Lys and His. The gaister charges are then added to the protein molecule and saved as PDBQT macro molecule. The Aldose along with Hydrogen bonds is as shown in Fig 1.

2.1.2 Preparing the Ligand file

Based on the purpose of the study, the essential phytochemicals obtained from *C. citratus* [1], such as Myrcene, Citral and Geraniol were considered as ligand molecules. The ligand files were prepared using Chemsketch [33]. The ligand files are then converted into PDB file format by adding all the hydrogen bonds to it using Bable. Adding hydrogen bonds is necessary for the uniform gaister charge distribution which is required during docking. In a docking procedure either ligand or receptor molecule can set free to rotate. The free rotation of either ligand or receptor

FIGURE 1: Aldose with Hydrogen Bonds

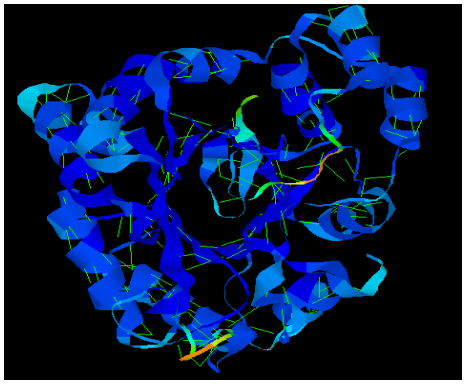
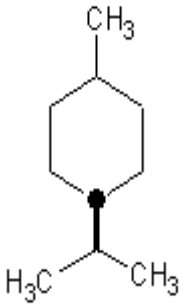


FIGURE 2: 7-methyl-3-methylene-1,6-octadiene(m)



provides the best execution of LGA in which one species is kept rigid and other allow rotating freely. In our analysis we have set the receptor molecule (Aldose) as rigid species and ligand species is set free to rotate around it. The torsion tree root (around which the ligand rotates) is then detected for all the three ligands. For Citral the torsion root is detected at carbon atom C6 where torsion root is represented by a black dot and allowable rotational bonds with dark black lines as shown in [Fig 3].

FIGURE 3: 3, 7-dimethyl-2, 6-octadienal (c) (g)

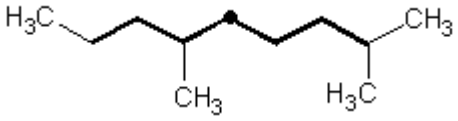
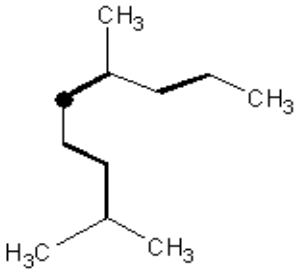


FIGURE 4: 3, 7-dimethyl-2, 6-octadien-l-ol



For Myrcene the torsion root detected at carbon atom C6 where the modified Myrcene with torsion root represented by a dot and one allowable rotational bond by black line as shown in [Fig 2]. The modified Geraniol is as shown in [Fig 4] with torsion root represented by a black dot and four allowable rotational bonds with dark black lines.

2.1.3 Preparing the Grid Parameter File

A grid is a system space under consideration. Grid is 3D space in which the receptor [1ADS] is embedded and a probe atom is placed at each grid point. An affinity is calculated for each type of atom in a substrate, mainly carbon, oxygen, nitrogen and hydrogen as well as grid of electrostatic potential either using a point charge of +1 as probe, or using Poisson – Boltzmann finite difference method such as DELPHI. Auto dock requires a pre calculated grid maps, one for each atom type present in the ligand. A grid map consist of 3D lattice of regularly spaced point, surrounding (either entirely or partially) the region of interest in the macromolecule. The grid size is selected such that it covers the entire molecule to provide extreme freedom of rotation to the ligand molecule.

2.2 Results and Discussion

Based upon the limited data and some earlier findings on Aldose reductase inhibitors obtained from natural sources such as flavonoids, coumarins, stilbenes, monoterpenes, and related aromatic compounds, it has been well acknowledged that plant derived extracts and phytochemicals are potential alternatives to synthetic inhibitors against Aldose reductase [33-37], although *in vivo* efficacy and clinical utility remain to be evaluated. In order to explore the inhibitory mechanism of natural compounds/phytochemicals towards Aldose reductase, bioinformatics plays a major role, where docking can be done using computer software to find out the binding sites and docking mechanisms. This provides a clear view on how the drug acts upon Aldose reductase and inhibits its activity. The results imply that methods designed to normalize Aldose reductase activity could be of significant benefit in the prevention and treatment of diabetic retinopathy [38]. From the docking results obtained, it can be concluded that the phytochemicals effectively docked into the binding site of Aldose reductase protein indicating that they are efficient drug compounds. The docking of Aldose reductase by myrcene showed best binding energy as compared to other two compounds viz., citral, geraniol. All the three components have more or less similar docking binding energies and so it might be expected that all the three active components can be used for synergistic inhibition on Aldose reductase activity under *in vivo* condition. These results suggest that active components from *C. citratus* would have some pharmacological actions against Diabetes mellitus. Further this may be confirmed by drug trials in animal models to find out the optimum dose and its efficiency in inhibiting Aldose reductase activity and reduce diabetic related complications.

In view of the above, it was felt that molecular docking of Aldose reductase activity inhibition by using the essential phytochemicals of *C. citratus* such as myrcene, citral and geraniol would be helpful to understand the underlying mechanism. This work would provide a clear understanding on the mode of action of the above mentioned plant components of *C. citratus* individually or synergistically against Aldose reductase and can be used as an inhibitory drug to reduce diabetic related complications. Based on the previous analysis made on variation in contents of essential oil *in vivo* and *in vitro* plants of *C. citratus* [1] we have concluded the percentage of essential oils viz Citral, Myrcene and Geraniol as shown in table- 2 which shows the % variation in concentration of essential oils in *in vivo* and *in vitro* (both long and short morphotypes) plants. Since Myrcene is most effective for Aldose activity inhibition, as concluded from Auto Dock results, obtaining Myrcene from *in vivo* plants is most efficient than obtaining it from *in vitro* plants for the inhibition of aldose reductase activity.

In order to run Auto dock, grid maps have to be calculated. The dimension of grid points in x y z, directions were fixed as 90, 90, 90 respectively, this covers the active site extensively and allows the ligand move without any constraint regarding the box size. Spacing between grid points was kept as 0.375 Å with a common grid center. In the docking of three ligands viz: Citral, Geraniol and Myrcene, three docking parameter files (DPF) were generated one for each ligand by a Python script that use the methods in Auto Dock. The script takes one pdbqs file, loops over the pdbq file and sets the name of the maps and the ligand in the parameter file. It also sets the Lamarckian Genetic Algorithm (LGA) to be used with a population size of 50 individuals. These 50 were calculated at 10 different runs (i.e. 10 dockings) and the runs had two stop criteria-

- A maximum of 1000330 energy evaluations.
- A maximum of 993 generations.

The ligands were set to start in a random position and conformation, the translations were set to have a maximum of 2 Å/step and the torsion, both had a maximum at 50°/step. The elitism number was set to 1. The mutation rate and the crossover rate were 0.02 and 0.80 respectively. The probability that an individual in the population will undergo a local search was set to 0.06 and the constraint used in the Pseudo-Solis and Wet's local search was set to a maximum of 300 iterations per search. The size of the local search space was 1.0 and the smallest step that the

local search could take before the ending was set to 0.01. This creates a docking parameter file for each docking in the directory.

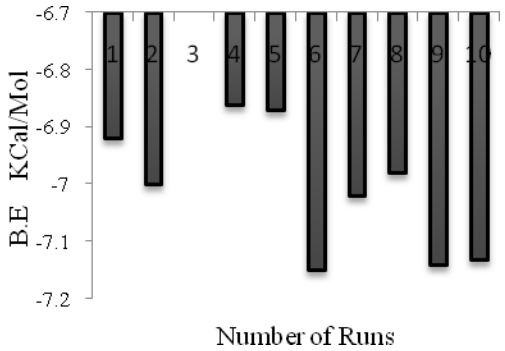
Docking results obtained showed the binding energy of all the three ligands (Citral, Geraniol and Myrcene) to the receptor molecule Aldose. Table 1 shows the binding energies of all the three ligands with receptor Aldose and it can be seen that Myrcene has the best binding energy whereas Citral and Geraniol are not much reliable for binding to receptor molecule with almost same binding energies.

TABLE 1: Binding energy and Mean Binding Energy

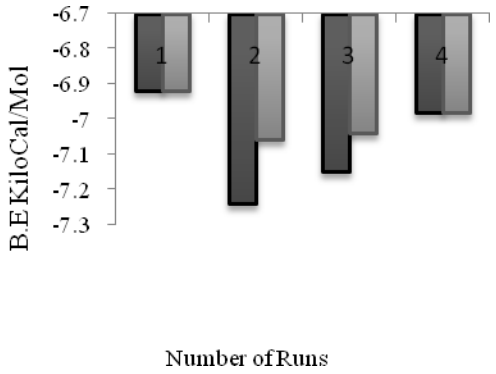
Ligand	**Binding Energy Kilo Cal/Mol**	**Mean Binding Energy Kilo Cal/Mol**
Geraniol	-7.93	-7.90
Citral	-7.24	-7.06
Myrcene	-8.76	-8.75

Based on the algorithm used, the docking result also showed, the Root Mean Square Deviations (RMSD) and Clustering data. Both, RMSD and clustering data is obtained based on the binding energy with respect to number of runs in which docking took place. Graph 1 shows the RMSD data of Citral and it can be seen that the binding energy of Citral varies in the range of -6.9 Kilo Cal/Mol to -7.1 Kilo Cal/Mol. Graph 2 shows the clustering of Citral with receptor ADS, wherein the Citral shows the best four clustering out of total 10 dockings. Both the lowest and mean binding energy of Citral clustering varies between -6.92 Kilo Cal/Mol to -6.98 Kilo Cal/Mol, with best lowest binding energy of -7.24 Kilo Cal/Mol and best mean binding energy of -7.06 Kilo Cal/Mol

Graph. 1- Citral RMSD

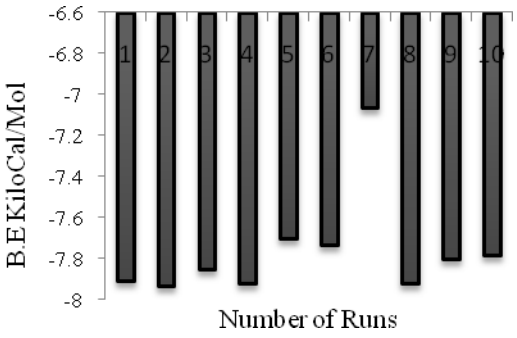


Graph. 2- Citral Clustering

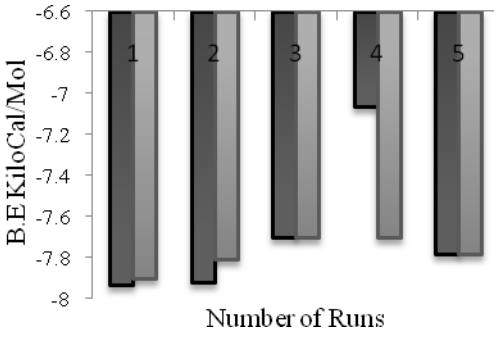


The RMSD and Clustering data of Geraniol is also generated as shown in Graph 3 and Graph 4 respectively. The binding energies in RMSD analysis of Geraniol [Graph 3] was found to be almost constant with respect to all the number of runs, varying in the range between -7.9 Kilo Cal/Mol to -7.7 Kilo Cal/Mol in all the 10 runs (10 Dockings). The clustering of Geraniol [Graph 4] shows the five best clustering out of total 10 runs with best lowest binding energy of -7.93 Kilo Cal/Mol and the best mean binding energy -7.9 Kilo Cal/Mol.

Graph. 3- Geraniol RMSD



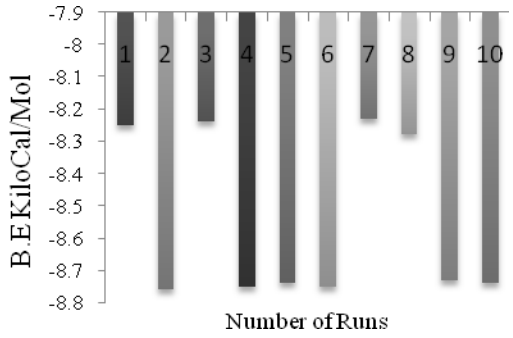
Graph. 4- Geraniol Clustering



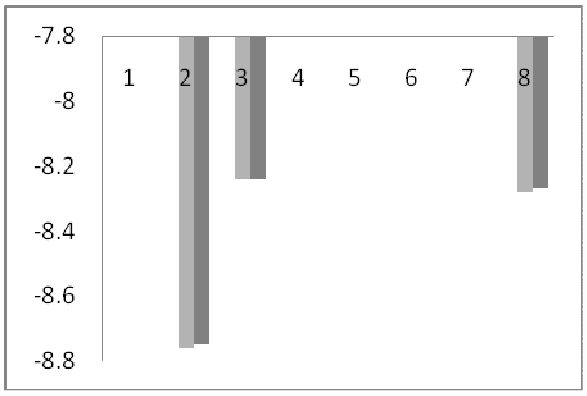
Similarly, the RMSD and clustering data of Myrcene is obtained as shown in [Graph 6] and [Graph 7]. Myrcene, in fact, showed the best binding energies both in RMSD as well as clustering

analysis. In RMSD analysis [Graph 6] the binding energy of Myrcene varies between the range of -8.2 Kilo Cal/Mol to -8.7 Kilo Cal/Mol in total of 10 runs (10 Dockings). The clustering of Myrcene [Graph 7] showed best three clustering out of total 10 with best lowest binding energy of -8.76 Kilo Cal/Mol and best mean binding energy of -8.75 Kilo Cal/Mol.

Graph. 5- Myrcene RMSD



Graph. 6- Myrcene Clustering



From the docking results obtained, it can be concluded that the compound Myrcene shows the best binding energy as compared to other two compounds (Citral and Geraniol) of *C. citratus* with respect to receptor Aldose. Based on the previous analysis made on variation in contents of essential oil *in vivo* and *in vitro* plants of *C.citratus* [1] we have concluded the percentage of essential oils viz Citral, Myrcene and Geraniol as shown in table- 2.

TABLE 2: Composition of Essential Oils of *C.citratus*

RI	Compound	Natural (%)	Long morphotype	Short morphotype
985	Myrcene	45.22	--	14.55
1242	Geraniol	1.59	1.67	2.18
1252	Citral	44.33	49.41	43.36

Table- 2 shows the % variation in concentration of essential oils in *in vivo* and *in vitro* (both long and short morphotypes) plants [1a]. Since Myrcene is of most important for Aldose activity inhibition, as concluded from Auto Dock results, obtaining Myrcene from *in vivo* plants is most efficient than obtaining it from *in vitro* plants, both long and short morphotypes for the inhibition of aldose reductase activity.

2.3 Conclusion

Overall the obtained data highlight the importance of phtytochemical from *Cymbopogon citratus* on the aldose reductase activity which will reduce diabetic complications for or great number of diabetes patient. It is in accordance with previously reported on the aldose redutase inhibitory activities of a number of phytochemicals.

Acknowledgements

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