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In Silico Docking Analysis of Natural Antioxidant from *Murraya koenigii* and Butylated Hydroxyanisole

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KEYWORDS

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A B S T R A C T

Superoxide is a common reactive form of oxygen that is formed when molecular oxygen gains a single electron. It is an anion with the chemical formula O_2^-, this in turn attacks susceptible biological targets, including nucleic acids, proteins and lipids. The extracellular superoxide dismutase (SOD3 or EC-SOD) is a major extracellular scavenger of the superoxide anion. It is a metalloenzyme, which requires Cu and Zn metal ions for its activation, which converts the superoxide radical (O_2^-) into oxygen (O2) or hydrogen peroxide (H_2O_2) and prevents cell damage. Antioxidants are man-made or natural substances that safely interact with free radicals to neutralize them and terminate the chain reaction before the damage of vital molecules. Synthetic antioxidants are artificial analogs of Natural Biomolecules. They are less reactive than natural antioxidants. One among the synthetic antioxidant is Butylated Hydroxyanisole (BHA) consisting of a mixture of two isomeric organic compounds, 2-tert-butyl-4-hydroxyanisole and 3-tert-butyl-4-hydroxianisole. BHA is able to stabilize free radical by sequestering them. By acting as free radical scavengers, further free radical reactions are prevented. *Murraya koenigii*commonly known as *meethineem*, belongs to the family *Rutaceae*, is native to India. The phytochemicals from the leaves of *Murraya koenigii* are Lutein, Tocopherol, Koenimbine, Carotene, O-methyl Murrayamine A, O-methyl Mahanine, Isomahanine, Bismahanine, Bispyrafoline, Euchrestine, Bismurrayafaline E, Mahanine, Mahanimbine which shows antioxidant activity. In this present study, natural antioxidants from the leaves of *Murraya koenigii* (Koenimbine and Mahanimbine) and synthetic antioxidant Butylated Hydroxyanisole (BHA) are docked against SOD3. The result shows that the natural antioxidants interacts more efficiently than the synthetic antioxidants.

Introduction

Oxidative stress is known to be involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, malignancies (Toshikazu *et al*., 2002). Oxidative stress releases harmful oxygen free radicals that attack biological molecules such as lipids, proteins, and DNA. Oxidative stress is defined as a "state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them." It not only causes hazardous events such as lipid peroxidation and oxidative DNA damage, but also physiologic adaptation phenomena and regulation of intracellular signal transduction (Toshikazu *et al*., 2002). Superoxides or free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction.

Superoxide dismutase (SOD) has the ability to scavenge this superoxide (O_2^-) and convert into normal oxygen molecules (O_2) and hydrogen peroxides (H_2O_2). SOD is present both inside and outside the cell membranes; one of the body's primary internal anti-oxidant defenses and plays a critical role in reducing the oxidative stress implicated in atherosclerosis and other life-threatening diseases. Three forms of superoxide dismutase are present in humans, in all other mammals, and most chordates. SOD1 is located in the cytoplasm, SOD2 in the mitochondria, and SOD3 is extracellular. The first is a dimer (consists of two units), whereas the others are tetramers (four subunits). SOD1 and SOD3 contain copper and zinc, whereas SOD2, the mitochondrial enzyme, has manganese in its reactive centre. Superoxide dismutase 3 (SOD3) or Extracellular superoxide dismutase (EC-SOD) is a major extracellular antioxidant enzyme, mainly located in the lymph, synovial fluid and plasma (Stralin *et al*., 1995). It requires copper and zinc metal ions for its activation. SOD3 protects nitric oxide (NO) bioactivity and decreases production of the strong oxidant, peroxynitrite (Demchenko *et al*., 2002).

An antioxidant is a molecule stable enough to donate an electron to a free radical and neutralize it, thus reducing its capacity to damage the DNA and other molecules. These antioxidants delay or inhibit cellular damage through their free radical scavenging property (Halliwell, 1995). This can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. In nature there are a wide variety of naturally occurring antioxidants which are different in their composition, physical and chemical properties (Naik, 2003). It's a known fact that citrus fruits (orange, lemons, etc.) containing a high amount of natural antioxidants. Vitamin A, C and E are the popular antioxidants, which play a crucial role in preventing peroxidation damage in the biological system (Fogliano *et al*., 1999; Muntana, 2003). Most phytochemicals and flavonoids have antioxidant activity and protect our cells against oxidative damage. Phytochemicals with antioxidant activity are allyl sulfides (onions, leeks, and garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), polyphenols (tea, grapes). Synthetic antioxidants are artificially manufactured, produced in laboratories. The most commonly used synthetic antioxidant is Butylated Hydroxyanisole (BHA). It consists of two isomeric organic compounds, 2-tert-butyl-4-hydroxyanisole and 3-tert-butyl-4-hydroxyanisole.It is prepared from 4-methoxyphenol and isobutylene. It has excellent solubility in fats and oils and heat

stable. It has been used in food packaging, animal feed, rubber, cosmetics and petroleum products to providing extended shelf life, with some restriction (LAM *et al*., 1979).

Murraya koenigii is one of the most important folklore, small aromatic, medicinal plant belonging to the family Rutaceae. The family Rutaceae has more than 150 genera and 1600 species (Muthumani *et al*., 2009).

It is commonly known as Kariveppilai in Tamil (Leela *et al*., 2013). The curry leaf tree is native to India, Sri Lanka, Bangladesh and the Andaman Islands (Suman *et al*., 2014).Of the 14 global species belonging to the genus *Murraya*, only two are available in India, namely, *Murrayakoenigii* and *Murrayapaniculata*. Of the two *Murrayakoenigii* is more popular due to its large spectrum of medicinal properties. *Murrayakoenigii* leaves have a slightly pungent, bitter and feebly acidulous taste and these characteristics are retained even after drying. Fresh and dried curry leaves are extensively used in South Indian culinary practices for seasoning and flavouring dishes (Pruthi, 1976). Different parts of the plant such as leaves, root, bark and fruit are known to possess various biological activities. Traditionally, this plant is used in Indian systems of medicine for a variety of ailments and also used as a tonic, stomachic, and carminative (Chevallier, 1996; Sivarajan and Balachandran, 1994; Muthumani *et al*., 2009). Leaf infusion is used in diarrhea and dysentery. Root and bark paste is useful for skin eruptions. The plant is also found to possess hepatoprotective activity (Sivarajan and Balachandran, 1994). *Murraya koenigii* has many biological activities such as antioxidant activity, anti-cancer, anti-diabetic, Anti-nociceptive, Lipid-lowering, Alzheimer's disease/Antiageing, Antibacterial activity (Harish *et al*., 2012). *Murraya koenigii* which possesses many bioactive compounds areLutein, Tocopherol, Koenimbine, Carotene, O-methyl Murrayamine A, O-methyl Mahanine, Isomahanine, Bismahanine, Bispyrafoline, Euchrestine, Bismurrayafaline E, Mahanine, Mahanimbine which shows antioxidant activity in leaves. In these compounds we select koenimbine and mahanimbine for in silico docking analysis to show that an antioxidant activity of *Murraya koenigii* is more efficient than BHA.

Materials and Methods

Uniprot

The Universal Protein Resource (UniProt) is a comprehensive resource for protein sequence and annotation data. The UniProt databases are the UniProt Knowledgebase (UniProtKB), the UniProt Reference Clusters (UniRef), and the UniProt Archive (UniParc). UniProt is a collaboration between the European Bioinformatics Institute (EMBL-EBI), the SIB Swiss Institute of Bioinformatics and the Protein Information Resource (PIR). Across the three institutes more than 100 people are involved through different tasks such as database curation, software development and support. (http://www.uniprot.org/)

Protein Data Bank

Protein Data Bank (PDB) format is a standard for files containing atomic coordinates. Structures deposited in the Protein Data Bank at the Research Collaboratory for Structural Bioinformatics (RCSB) are written in this standardized format. However, those actually creating PDB files should consult the definitive description

(http://www.rcsb.org/pdb/info.html#File_Formats_and_Standards).

Rasmol

This program is a simple, yet powerful tool, which enables you to visualize a molecule in "3-Dimensional space". Since we actively manipulate the computer mouse to rotate the molecule in computer space, we develop a sense of the 3-dimensionality of the molecule. Through RasMol, you can also change the display format of the molecule to display different features of the molecule (Shannon Colton, 2006).

Pubchem

PubChem is designed to provide information on biological activities of small molecules, generally those with molecular weight less than 500 Daltons. PubChem's integration with NCBI's Entrez information retrieval system provides sub/structure, similarity structure, bioactivity data as well as links to biological property information in PubMed and NCBI's Protein 3D Structure Resource. (http://pubchem.ncbi.nlm.nih.gov/)

ACD/Chemsketch

ChemSketch, freeware from ACD Labs, is a chemical structure drawing tool. Two-dimensional chemical structures are the common representation in textbooks and other print materials in chemistry, biology, and the health sciences. They display the interconnectivity of atoms in the structure. Knowing how to draw structures is required to understand them and helpful as they are converted into three-dimensional structures. Forming a three-dimensional mental image from a two-dimensional drawing is an advantage for scientists. Chime and RasMol are great aids to help develop this (Scott *et al*., 2004)

Open Babel

Open Babel is free software, a chemical expert system mainly used for converting chemical file formats (Boyle *et al*., 2011). Open Babel is a chemical toolbox designed to speak the many languages of chemical data. It's an open, collaborative project allowing anyone to search, convert, analyze, or store data from molecular modeling, chemistry, solid-state materials, biochemistry, or related areas.

Autodock

AutoDock is an automated procedure for predicting the interaction of ligands with bio macromolecular targets. The motivation for this work arises from problems in the design of bioactive compounds, and in particular the field of computer-aided drug design. Progress in bio molecular x-ray crystallography continues to provide important protein and nucleic acid structures (Garrett *et al*., 2012).

Pymol

PyMOL has become a capable molecular viewer with support for animations, high−quality rendering, crystallography, and other common molecular graphics activities. It has been be adopted by many hundreds (perhaps even thousands) of scientists spread over thirty countries. However, PyMOL is still very much a work in progress, with development expected to continue for years to come (Warren and Sarina, 2004).

Results and Discussions

Protein preparation

Sequence retrieval

The sequence of extracellular superoxide dismutase (EC-SOD or SOD3) is retrieved

from uniprot database and sequence accession number is P08294 (Human).

Structure Retrieval

The structure of the protein (SOD3) is prepared from PDB data base (PDB; http://www.rcsb.org/pdb/). Protein Data Bank has information about the 3D structures of proteins, nucleic acids, and complex assemblies that help to understand all aspects of biomedicine and agriculture, from protein synthesis to health and disease. The structure of superoxide dismutase3 (SOD3) is downloaded from PDB in pdb format and stored.

RASMOL View

The three dimensional structure of extracellular superoxide dismutase (EC-SOD3 or SOD3) is viewed using RasMol. RasMol is a computer program written for molecular graphics visualization intended and used primarily for the depiction and exploration of biological macromolecule structures, such as those found in the Protein Data Bank(Roger Sayle *et al*., 1995).

Table.1 Compounds 2D and 3D structure

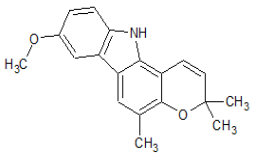
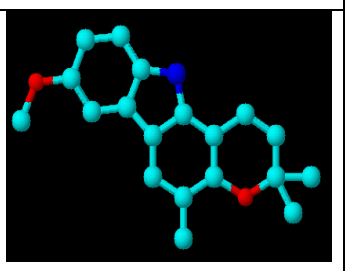
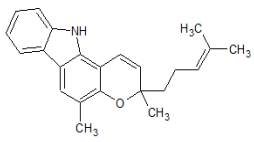
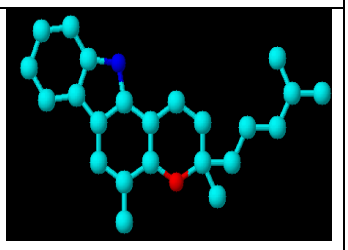
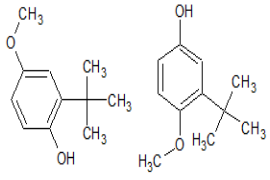
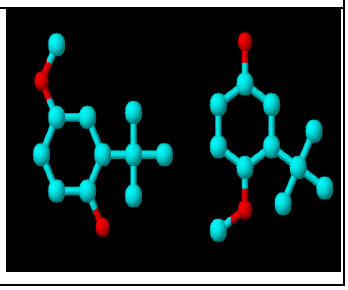
S.No	COMPOUNDS	MOLECULAR FORMULA	2D STRUCTURE	3D STRUCTURE
1	Koenimbine	$C_{19}H_{19}NO_2$		
2	mahanimbine	$C_{23}H_{25}NO$		
3	Synthetic BHA	$C_{11}H_{16}O_2$		

Table.2 Docking interaction between SOD3 and Koenimbine

SOD3		Koenimbine	Distance (Å)	Docking energy (Kcal/Mol)
Residue	**Atom**			
HIS-153	ND1	O	2.1	-4.89

Table.3 Docking interaction between SOD3 and Mahanimbine

SOD3		Mahanimbine	Distance (Å)	Docking energy (Kcal/Mol)
Residue	**Atom**			
PHE-84	O	H	2.1	-5.33

Table.4 Docking interaction between SOD3 and synthetic BHA

SOD3		BHA	Distance (Å)	Docking energy (Kcal/Mol)
Residue	**Atom**			
ALA-132	O	H	2.1	-3.85

Fig.1 Murraya koenigii (Curry leaves)

Fig.2 Crystal structure of SOD3

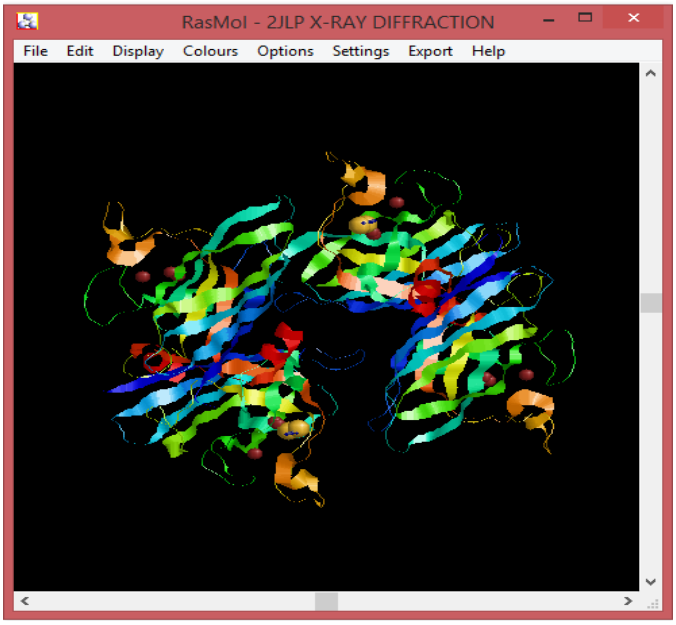
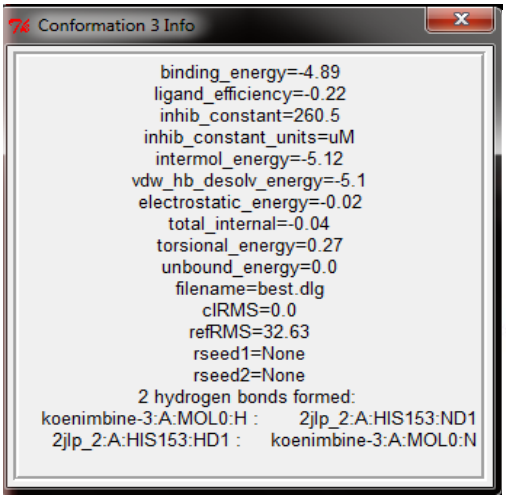
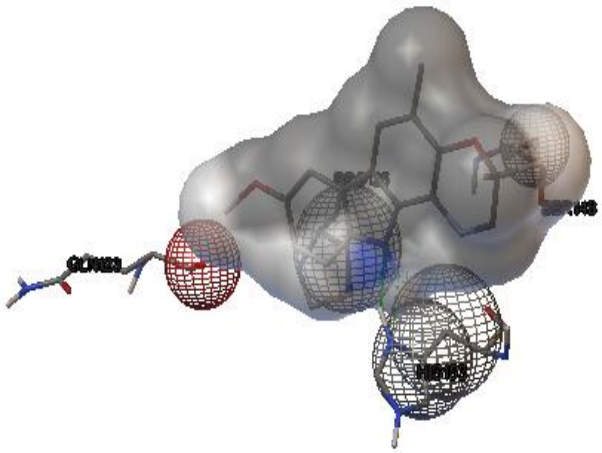
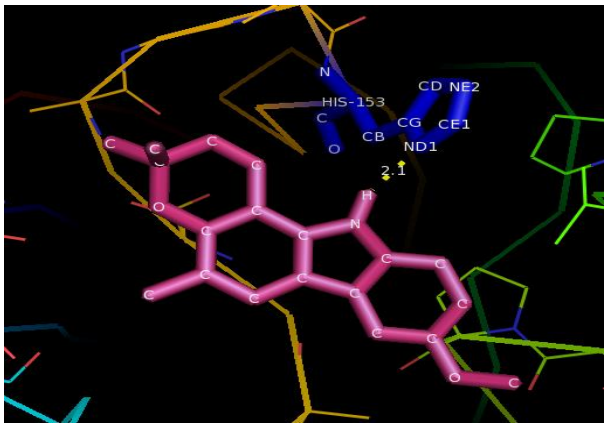


Fig.3 Docking analysis between koenimbine and SOD3. (a)Binding score (b) Interaction between SOD3 and koenimbine is visualized using Autodock (c) Hydrogen bond forms between SOD3 and koenimbine is visualized using Pymol



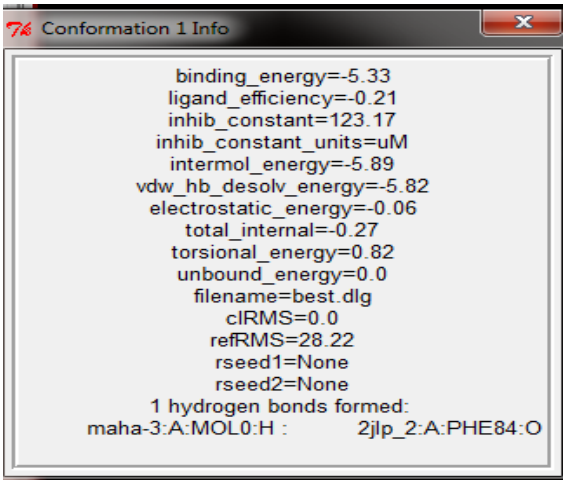


(a) (b)

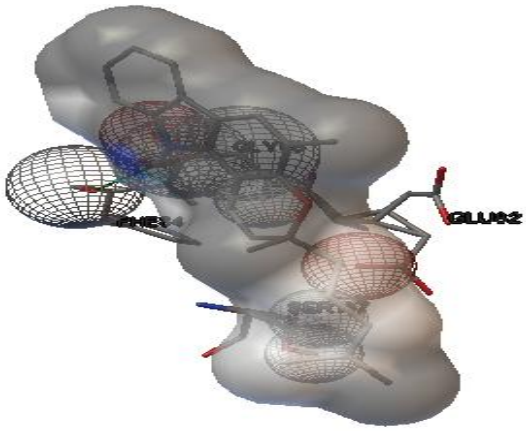


(c)

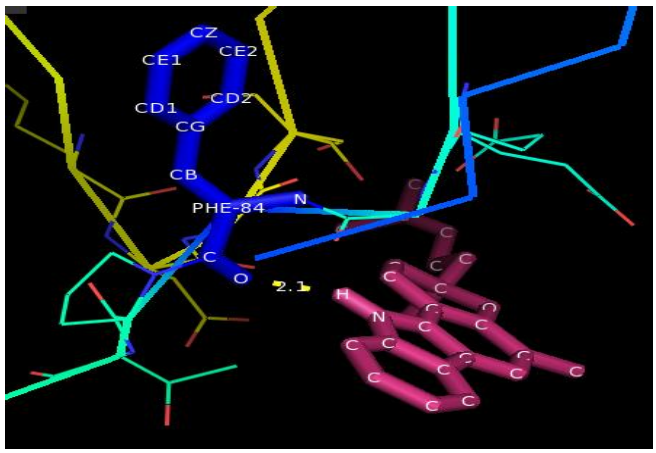
Fig.4 Docking analysis between mahanimbine and SOD3. (a)Binding score (b) Interaction between SOD3 and mahanimbine is visualized using Autodock (c) Hydrogen bond forms between SOD3 and mahanimbine is visualized using Pymol



(a)

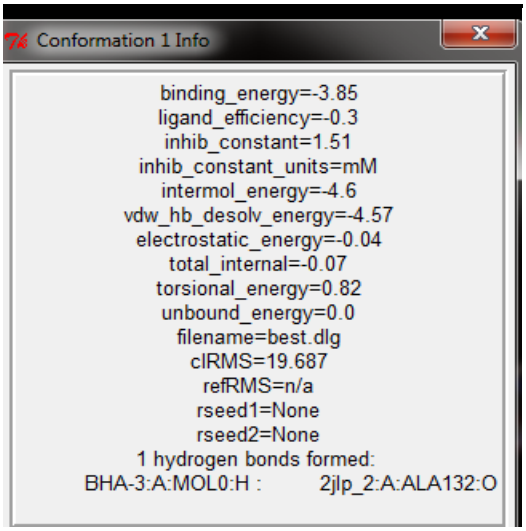


(b)

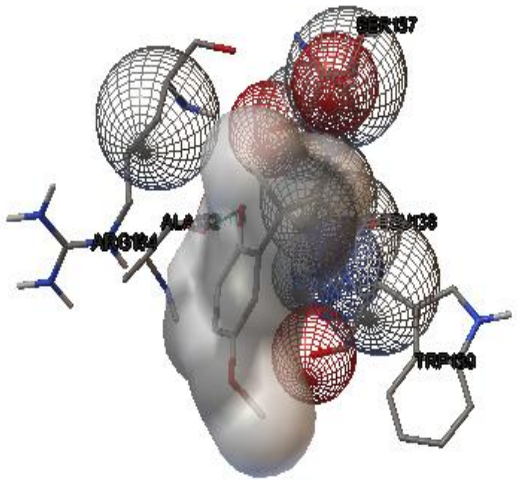


(c)

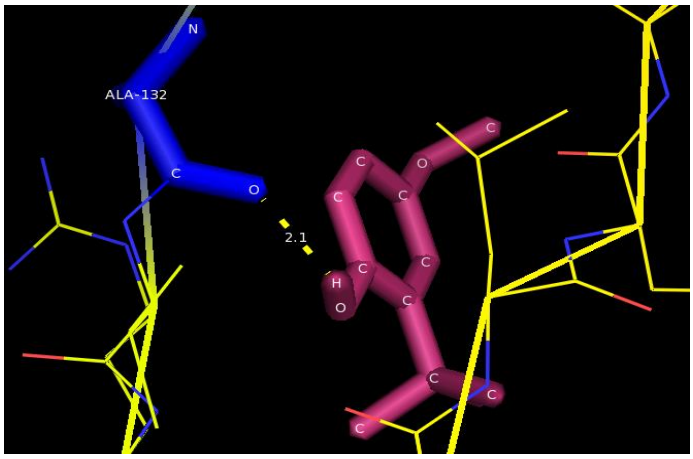
Fig.5 Docking analysis between BHA and SOD3. (a)Binding score (b) Interaction between SOD3 and synthetic BHA is visualized using Autodock (c) Hydrogen bond forms between SOD3 and synthetic BHA is visualized using Pymol



(a)



(b)



(c)

Ligand Preparation

PUBCHEM

The phytochemicals from the leaves of *Murraya koenigii*, koenimbine and mahanimbine was selected for the docking analysis along with synthetic butylated hydroxyanisole (BHA) antioxidant compound.

ACD/CHEMSKETCH

The 2-dimensional structure of compounds are cleaned and optimized in 3-dimensional structure by using ACD/ChemSketch tool. It's a chemical drawing tool that can perform 2D cleaning, 3D optimization and viewing of chemical structures.

These data are saved as a molecular format file (MDL MOL format). The molecular

format converter tool (Open Babel) is used to converter this file into the PDB format and is used during docking analysis. The structure and molecular formula of compounds was shown in Table 1.

Docking Analysis

The phytochemical compounds of *Murraya koenigii* and BHA were docked against SOD3. The graphical user interface program "auto-dock tool" was used to prepare, run and analyze the docking simulations. The best ligand-receptor structure from the docked structures was chosen based on the lowest binding energy and minimal solvent accessibility of the ligand. The interaction between the ligands and protein is visualized and the distance between the donor and acceptor atoms was measured using pymol.

From the present in silico docking analysis of koenimbine and mahanimbine from the leaves of *Murraya koenigii* and synthetic butylated hydroxyanisole (BHA) against superoxide dismutase 3 (SOD3), shows 1 hydrogen bond each with -4.89, -5.33 and -3.85 respectively. However phytochemicals from the leaves of *murraya koenigii* forms strong hydrogen bonds and similar interaction with SOD3, than the synthetic butylated hydroxyanisole (BHA). Hence these bioactive compounds can be used as potent active drug to reduce oxidative stress.

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