IN-SILICO ANTIOXIDANT ACTIVITY OF BIOACTIVE MOLECULES OF CURRY LEAVES (MURRAYA KOENIGII) (L.)

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ABSTRACT:

The antioxidant activity of curry leaves is of interest because oxidative stress is implicated in a variety of diseases, including cancer, heart disease, and neurodegenerative disorders. By scavenging free radicals and inhibiting lipid peroxidation, antioxidants can help to protect cells from damage and reduce the risk of these diseases. Overall, *in-silico* studies suggest that curry leaves and their bioactive compounds have potent antioxidant activity. The objective of the study was to assess the antioxidant activity of the bioactive molecules from *Murraya koenigii* and anticipate the toxicity by in-silico research. iGEMDOCK software was used to assess the antioxidant activity. ProTox II software was used to conduct the toxicity investigations. Osthol (-81.4863, -81.9046) kcal/mol, Mukoenine A (-81.0417, -78.9941) kcal/mol, Murrayone (-80.0731, -78.9453) kcal/mol, Kaempferol (-97.0774, -89.3534) kcal/mol, Apigenin (-89.5291, -108.704) kcal/mol were found to have the highest binding energies with SOD and GPx proteins, respectively when compared with Ascorbic Acid (-81.132, -98.917) kcal/mol. All the above-mentioned compounds have low levels of hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. The bioactive molecules of *Murraya koenigii* have a potent antioxidant effect and low level of toxicity that gives valuable insight for optimizing lead molecules to combat free radical generation or oxidation.

Keywords: *Murraya koenigii*, iGEMDOCK, ProTox II, In-silico, Antioxidant

INTRODUCTION

The small aromatic tree *Murraya koenigii* Spreng, which is a member of the Rutaceae family, is known for its curly leaves. Native to India, this tree is tropical to subtropical in nature. Just two species—*Murraya koenigii* and *M. paniculata*—of the genus Murraya's fourteen worldwide species are found in India. Because of its wide range of therapeutic benefits, *M. koenigii* is the more well-known of the two. The mildly bitter, slightly acidulous, and slightly pungent flavor of *M. koenigii* leaves persists even after drying. In South Indian cooking, curry leaves (fresh and dried) are widely used to flavor and season food[1]. It is known that different plant parts, including the fruit, bark, roots, and leaves, have distinct biological functions. This plant has long been utilized in Indian medical systems as a tonic, stomachic, and carminative for a range of illnesses [2- 4].

Flavonoids, coumarins, and carbazole alkaloids are the main chemical components of the plant that have been identified previously [5]. In laboratory animals, leaf extracts from *M. koenigii* show hypoglycemic and hypolipidemic effects[6-8].It has also been reported that *M. koenigii's* carbazole alkaloids and methanolic extracts have anti-oxidative [9–11], anti-diarrheal, and anti-trichomonal properties[12, 13]. In ob/ob mice, *M. koenigii* leaf extracts decreased blood glucose and cholesterol levels[14].*M. koenigii* leaf methanolic extract has immunomodulatory [15] and anti-inflammatory properties[16]. Human leukemia cells undergo apoptosis when exposed to mahanine, a purified carbazole alkaloid from *M. koenigii* foliage [17-19] [20]. Antioxidants are a useful tool for preventing such oxidative damage.

Antioxidants include carotenoids, flavonoids, and vitamins A, C, and E. Flavonoids, also known as polyphenols, are frequently found in plants as glycosides [21]. According to studies, flavonoids work as antioxidants by preventing lipid peroxidation, scavenging free radicals and active oxygen, deactivating lipoxygenase, and chelating iron ions[22] [23].

Living organisms have evolved a system for the scavenging of free radicals and reactive species. Superoxide dismutase (SOD) carried out the removal of $O_2^{\bullet-}$. In another step, the hydrogen peroxide generated is eliminated by glutathione peroxidase(GPx) and catalase system. Various isoenzymes of SOD are indulged in the scavenging activity of free radicals. The first isoenzyme is present in the mitochondria and is Mn^{2+} dependent whereas another isoenzyme is available in the cytoplasm and is dependent on Cu. Another extracellular Cu-Zn-dependent isoenzyme is also reported. Two different mechanisms have been reported for the SOD action that restricts the transformation 1) It majorly acts at the cell membrane level and eliminates or prevent the generation of radical that can result from the peroxidation of lipid and leads to the chain of extra-nuclear and nuclear events, ultimately cause transformation. This concept explains the model of a membrane that mediates chromosomal aberration, 2) It has been reported that oxygen radicals could be generated in a growth medium and act as a promoter of transformation that will be removed by SOD[24,25].Superoxide dismutases (SODs) are the main antioxidant defense mechanisms against $O_2^{\bullet-}$. In mammals, there are three different forms of SOD: cytoplasmic Cu/ZnSOD (SOD1), mitochondrial MnSOD (SOD2), and extracellular Cu/ZnSOD (SOD3). Each type of SOD requires a catalytic metal (Cu or Mn) to be activated[26].

A cytosolic enzyme called glutathione peroxidase (GPx) catalyzes the conversion of peroxide radicals to alcohols and oxygen as well as the reduction of hydrogen peroxide to water and oxygen. Eight isozymes (GPx1–8) that make up the glutathione peroxidase family catalyze the reduction of hydroperoxides, lipid hydroperoxides, and hydrogen peroxide by reduced glutathione (GSH). Five of the eight glutathione peroxidases (GPx1-4, GPx6) contain selenium [27].

A crucial tool in computer-assisted drug design and structural molecular biology is molecular docking. Predicting the predominant binding mode(s) of a ligand with a protein that has a known three-dimensional structure is the aim of ligand-protein docking. Effective docking techniques use a scoring function that appropriately ranks candidate dockings and efficiently search high-dimensional spaces. Docking is a valuable tool for lead optimization because it can be used to virtually screen large compound libraries, rank the results, and suggest structural hypotheses about how the ligands inhibit the target. It can occasionally be difficult to interpret the outcomes of stochastic search techniques, and configuring the input structures for the docking is just as crucial as the docking process itself [28].

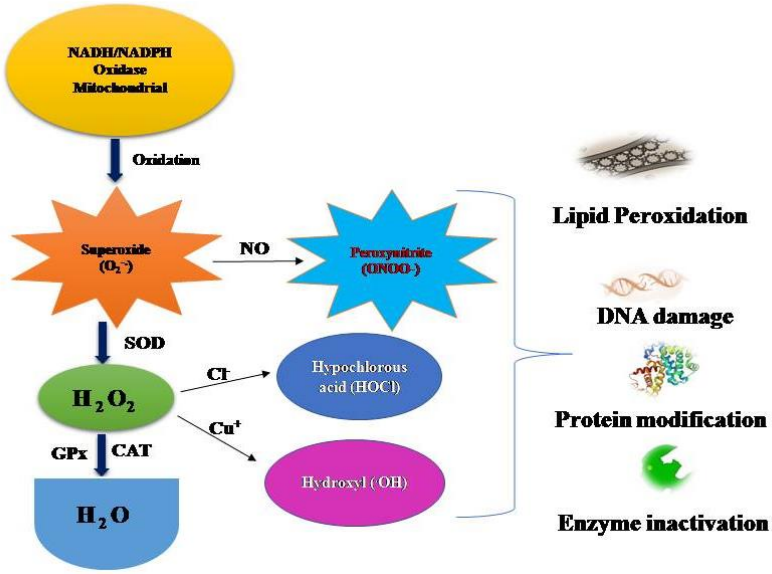
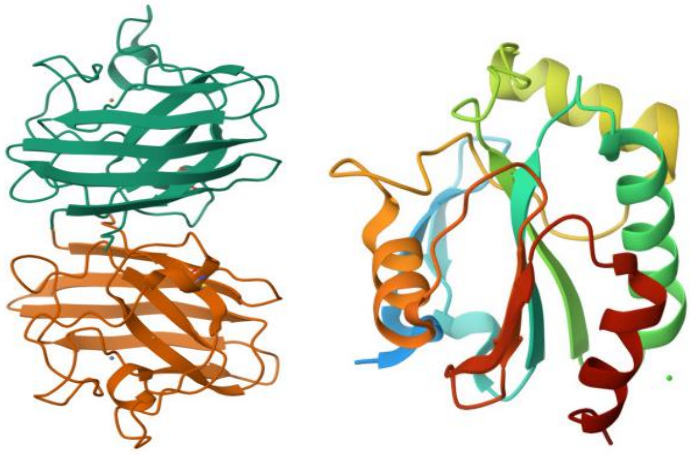


Fig 1. Overview of antioxidant action point and ROS kinds and sources.

METHODOLOGY

Docking investigations were conducted utilizing the iGEMDOCK suit v 2.1 between the bioactive compounds (ligand) from the Murraya koenigii plant and the proteins (PDB ID: 1CB4 and 2P31)[30, 31][34]. For the in-silico molecular docking research, the three-dimensional structures of the necessary proteins, SOD (PDB ID: 1CB4) and GPx (PDB ID: 2P31), have been retrieved at 2.30 Å and 2.00Å resolution from the Protein Data Bank (https://www.rcsb.org).



(A) (B)

Fig 2. Structure of Proteins (A) SOD (PDB ID: 1CB4) (B) GPx (PDB ID: 2P31)

Ligand Assembling:

The compounds generated in the *Murraya koenigii* for molecular docking profiling were used to create the bioactive ligands. The bioactive compounds were extracted from the PubChem database. Openbabel (https://openbabel.org) was used to convert the three-dimensional conformers of the molecules into Mol file (.mol) format for the protein-ligand interaction[34].

Docking Procedure:

iGEMDOCK offers a complete VS environment, from setup to post-screening analysis, including drug interactions. To start, iGEMDOCK has interactive interfaces for setting up the screening chemical library and the binding area of the target protein. The docking software iGEMDOCK was utilized to perform docking for proteins and ligand binding interaction in order to enhance antioxidant activity. The integrated virtual screening (VS) environment with pharmacological interactions is offered by iGEMDOCK, starting with preparation and ending with post-screening analysis. By determining the pharmacological interactions from screening compounds without depending on the experimental data of active compounds, iGEMDOCK provides biological insight. Subsequently, iGEMDOCK produces profiles of van der Waals (V), hydrogen bonding (H), and electrostatic (E) interactions between molecules and proteins. Based on these profiles and compound structures, iGEMDOCK clusters the screening compounds for post-screening analysis and infers pharmacological interactions[32] [34].

Furthermore, protein-ligand interaction profiles of electrostatic, hydrogen-bonding, and Van der Waals binding energies are generated by iGEMDOCK. Based on this information and the structures of bioactive molecules, iGEMDOCK generates pharmacological interactions and gathers ligands for post-screening analysis. Lastly, by integrating the energy-based scoring function of GEMDOCK with the pharmacological interactions, iGEMDOCK ranks and visualizes the screening molecules [33] [34].

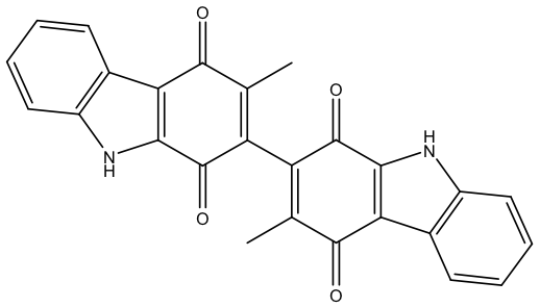
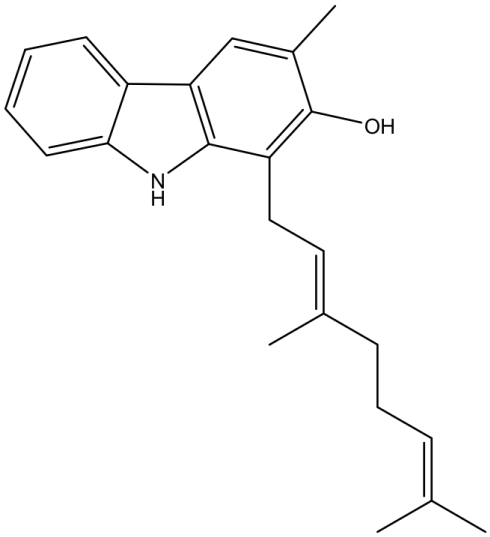
Dock Profiling:

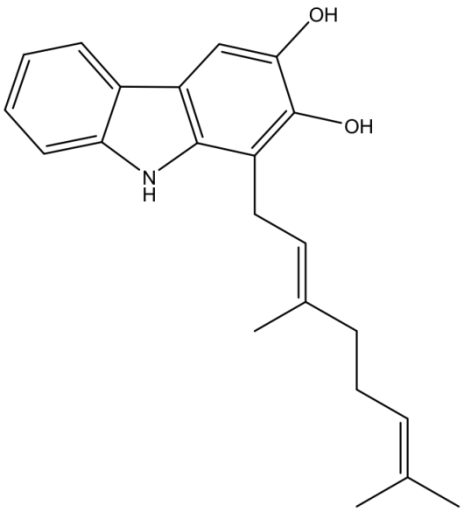
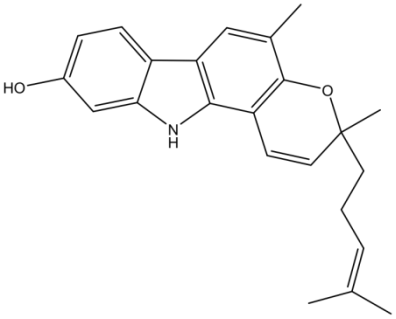
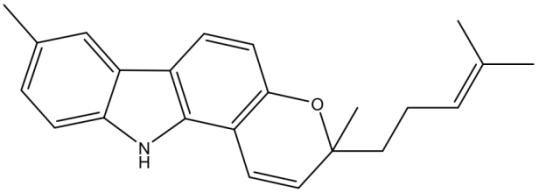
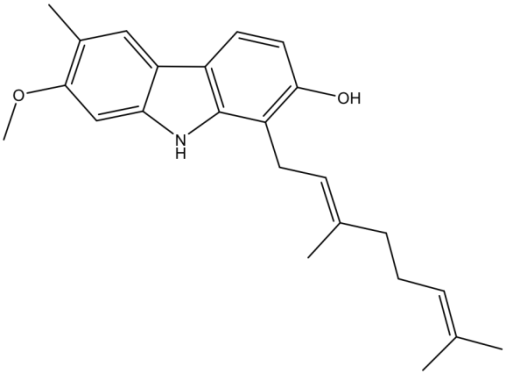
The protein and ligand molecules were assigned for bonds, bond orders, explicit hydrogen charges, and flexible torsions during the docking process using the iGEMDOCK software. When it came to docking accuracy, the genetic algorithm (GA) parameters were maintained at the standard docking population size of 200, generations of 70, and number of solutions of 2. The ligand intra-energy of hydrophobic and electrostatic preferences was maintained at 1.00 in the docking scoring functions. Proteins and inhibitors underwent docking, which produced binding affinities in kcal/mol and docking run times. The best inhibitor is the one with the lowest energy [34].

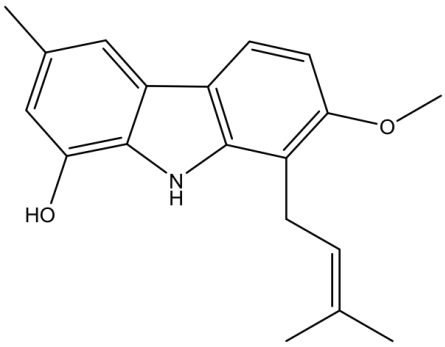
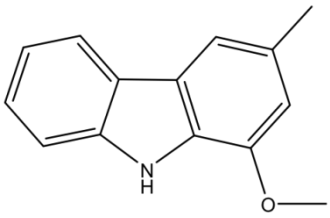
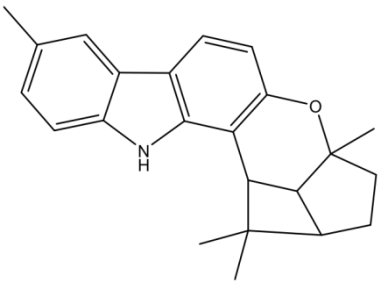
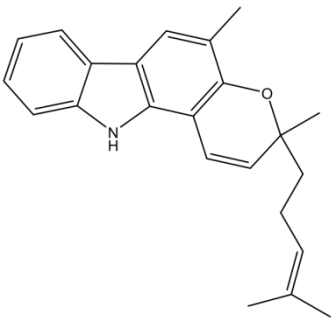
RESULTS:

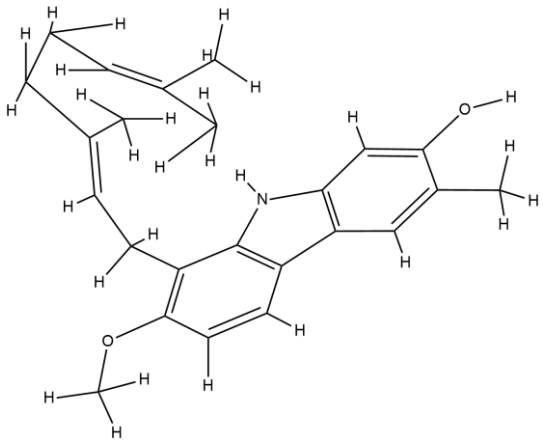
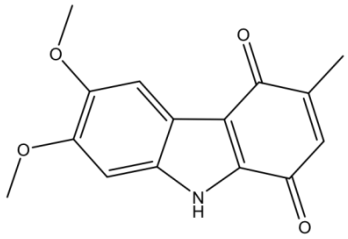
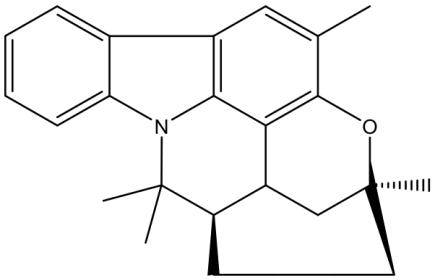
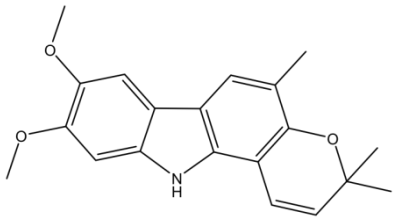
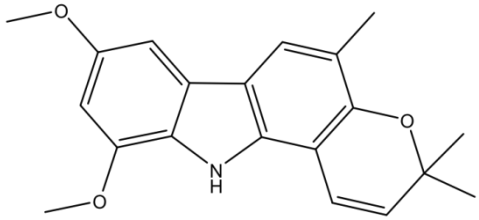
The ligands Osthol, Mukoenine A, Murrayone, Kaempferol, Apigenin, and Ascorbic Acid were docked with the SOD and GPx proteins using the iGEMDOCK software suite. The molecules' docked score, binding energy, molecular weight, log P, hydrogen bond donor, and hydrogen bond acceptor are all shown in Table 1. The ligands Osthol (-81.4863, -81.9046) kcal/mol, Mukoenine A (-81.0417, -78.9941) kcal/mol, Murrayone (-80.0731, -78.9453) kcal/mol, Kaempferol (-97.0774, -89.3534) kcal/mol, Apigenin (-89.5291, -108.704) kcal/mol, and Ascorbic Acid (-81.132, -98.917) kcal/mol were all occupied the binding pockets with SOD and GPx proteins. Molecular weight, log P, HBD, HBA for above compounds are Osthol (244.29gm/mol, 3.34, 0, 3), Mukoenine A (265.35gm/mol, 4.38, 2, 1), Murrayone (258.27gm/mol, 2.56, 0, 4), Kaempferol (286.24gm/mol, 1.58, 4, 6), Apigenine (270.24gm/mol, 2.11, 3, 5), and Ascorbic Acid (176.12gm/mol, -1.28, 4, 6) respectively. Table 2 showed the toxicity profile of bioactive compounds from *Murraya koenigii*.

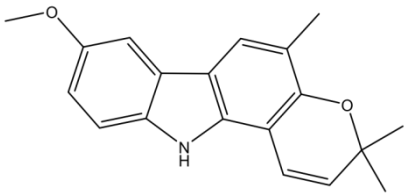
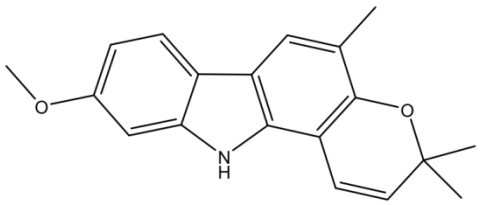
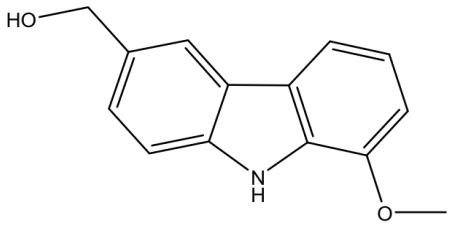
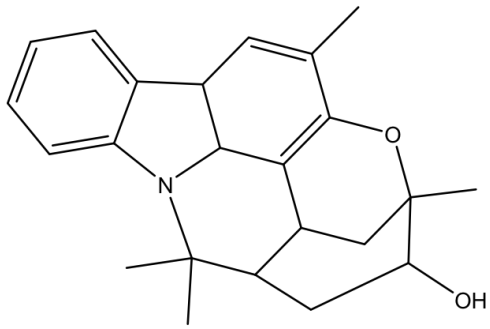
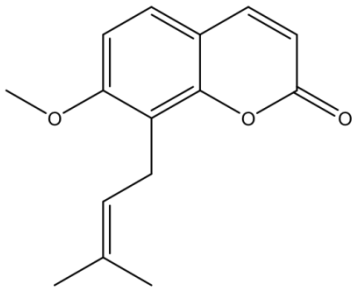
TABLE 1- Post-screening analysis of Phytomolecules from *Murraya koenigii* (L.)

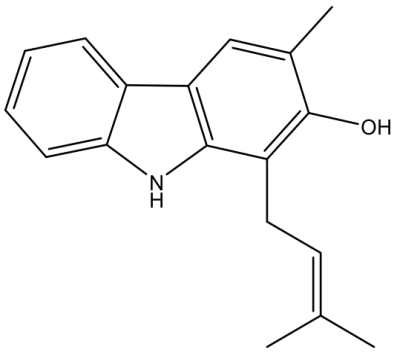
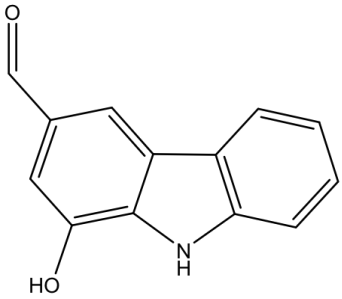
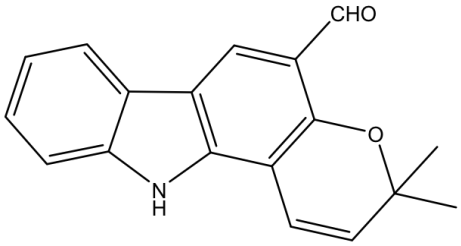
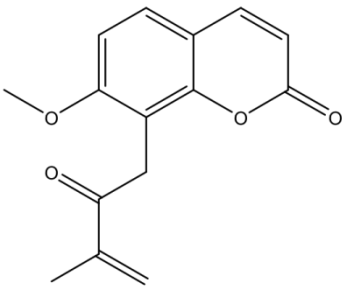
SN	Phytomolecules	Total Binding Energy (1cb4)	Total Binding Energy (2p31)	MW (g/mol)	Log P <5	HBD <5	HBA <10
1	Bismurrayaquinone A 	-116.513	-98.1197	420.42	3.73	2	4
2	Mahanimbinol 	-103.088	-86.8477	333.47	5.82	2	1

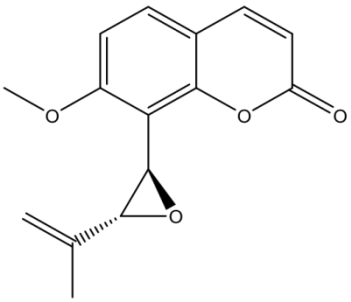
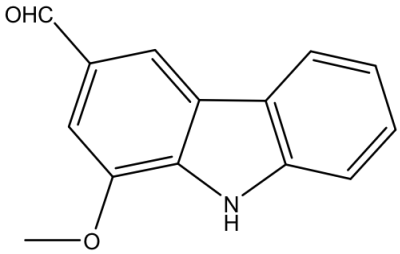
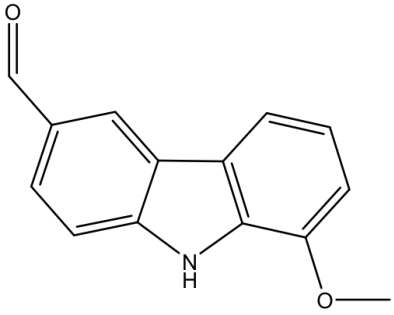
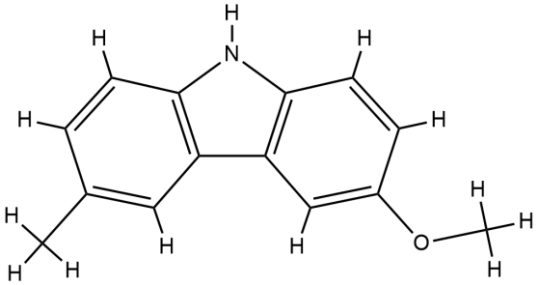
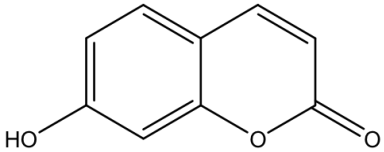
3	Mukoenine B 	-100.778	-88.1113	347.45	5.35	2	2
4	Mahanine 	-99.9027	-88.6737	347.45	5.20	2	2
5	Mahanimbicine 	-99.4095	-87.3616	331.45	5.63	1	1
6	Murrayanol 	-97.5276	-93.05	363.49	5.71	2	2

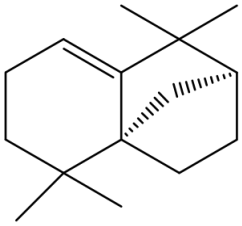
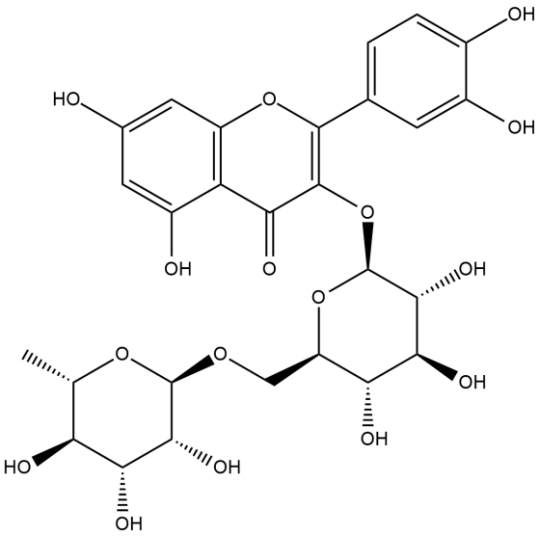
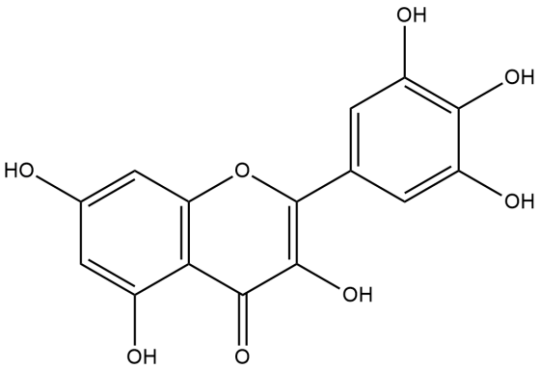
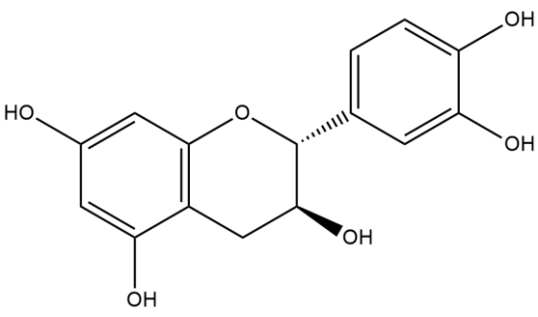
7	Murrayafoline B 	-96.3801	-97.3629	295.38	4.34	2	2
8	Murrayafoline A 	-94.4274	-83.5329	211.26	3.31	1	1
9	Bicyclomahanimbicine 	-91.6938	-75.4899	331.45	5.26	1	1
10	Mahanimbine 	-90.8303	-84.0423	331.45	5.62	1	1

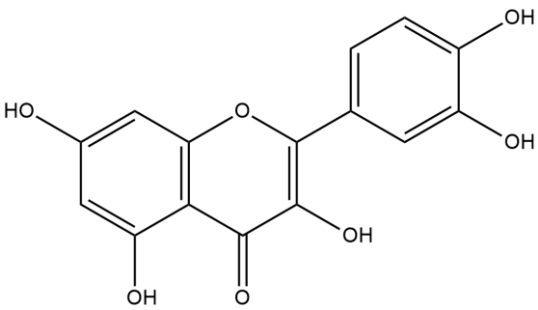
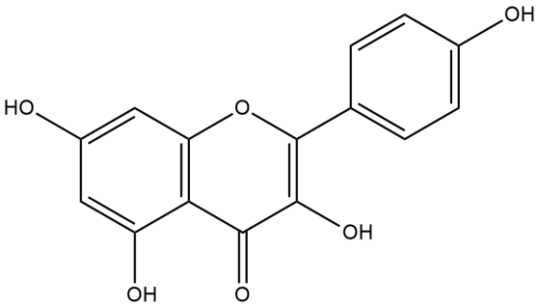
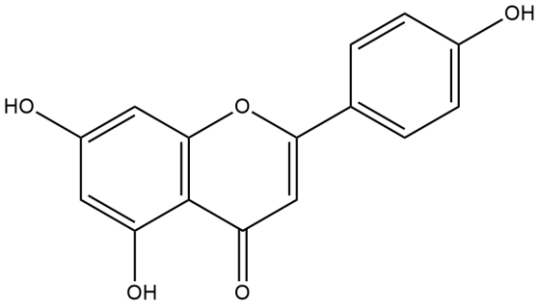
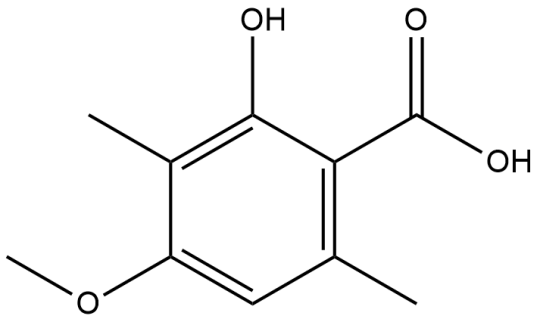
11	Euchrestine B 	-86.8592	-95.4025	363.49	5.75	2	2
12	Koenigine 	-86.695	-96.8815	309.36	3.78	2	3
13	Murrayazoline 	-86.599	-72.2561	331.45	4.94	0	1
14	Koenigicine 	-85.5764	-105.742	323.39	4.12	1	3
15	Mukonicine 	-85.4161	-93.451	323.39	4.16	1	3

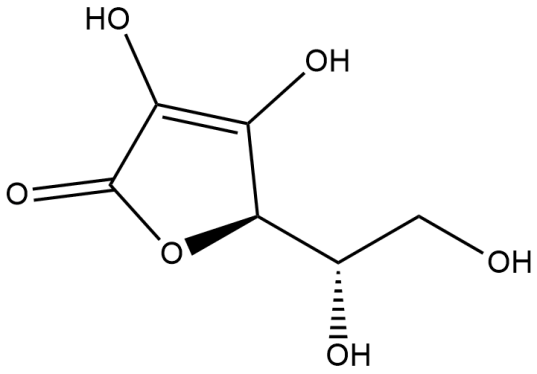
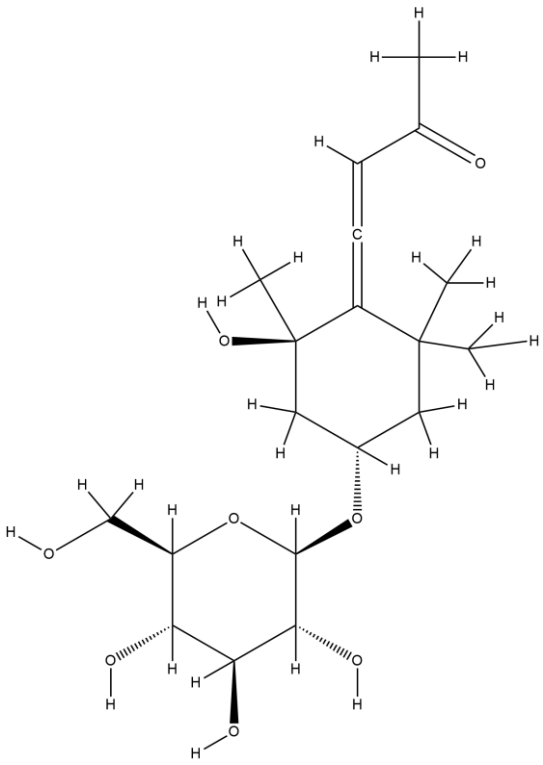
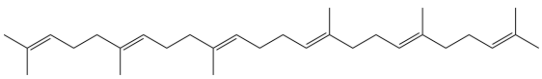
16	Koenimbine 	-85.1705	-75.9794	293.36	4.15	1	2
17	O-Methylmurrayamine A 	-84.0622	-77.1932	293.36	4.16	1	2
18	Mukoline 	-83.7169	-94.1819	227.26	2.54	2	2
19	Murrayazolinol 	-82.1961	-80.5304	347.45	4.15	1	2
20	Osthol 	-81.4863	-81.9046	244.29	3.34	0	3

21	Mukoenine A 	-81.0417	-78.9941	265.35	4.38	2	1
22	Mukonal 	-80.9036	-91.8855	211.22	2.36	2	2
23	Murrayacine 	-80.8233	-94.4165	277.32	3.56	1	2
24	Murrayone 	-80.0731	-78.9453	258.27	2.56	0	4

25	Phebalosin 	-79.4309	-87.8419	258.27	2.67	0	4
26	Murrayanine 	-78.8153	-90.0005	371.52	2.69	1	2
27	Mukolidine 	-77.9448	-82.97	225.24	2.70	1	2
28	Glycozoline 	-71.4867	-86.5918	211.26	3.29	1	1
29	Umbelliferone 	-69.2738	-89.6325	162.14	1.51	1	3

30	Isolongifolene 	-60.2781	-56.9009	204.35	4.54	0	0
31	Rutin 	-124.755	-114.454	610.52	-1.29	10	16
32	Myricetin 	-107.251	-100.595	318.24	0.79	6	8
33	Catechin 	-105.019	-85.9009	290.27	0.85	5	6

34	Quercetin 	-99.656	-91.1216	302.24	1.23	5	7
35	Kaempferol 	-97.0774	-89.3534	286.24	1.58	4	6
36	Apigenin 	-89.5191	-108.704	270.24	2.11	3	5
37	2-Hydroxy-4-methoxy-3,6-dimethylbenzoic acid 	-82.9651	-89.4158	196.2	1.70	2	4

38	Ascorbic acid 	-81.132	-98.917	176.12	-1.28	4	6
39	Icariside B1 	-107.338	-100.333	386.44	-0.15	5	8
40	Squalene 	-92.1756	-96.5304	410.72	9.38	0	0

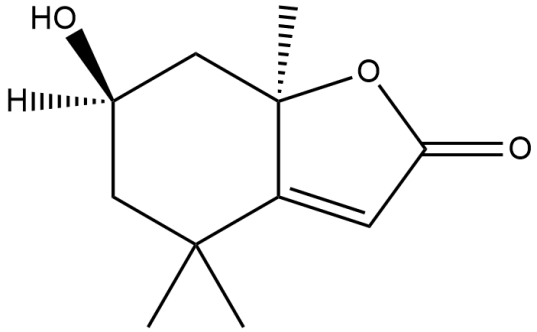
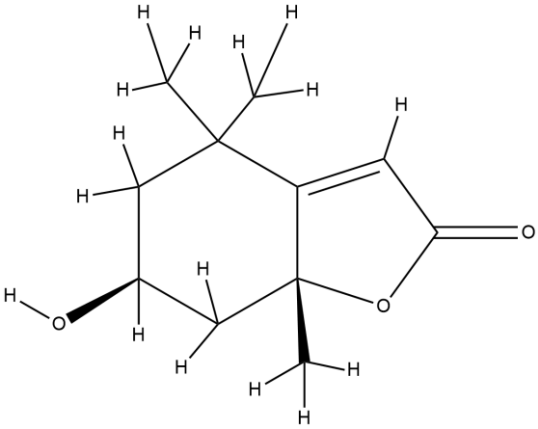
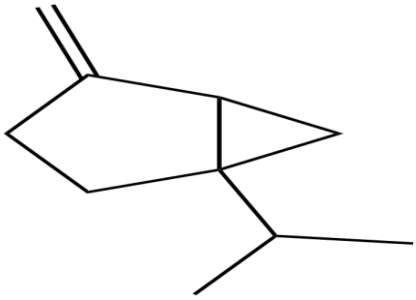
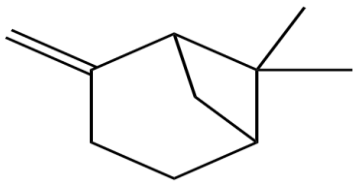
41	Epiloliolide 	-74.5368	-66.9872	196.24	1.53	1	3
42	Loliolide 	-65.1192	-69.8825	196.24	1.53	1	3
43	Sabinene 	-49.9988	-61.5002	136.23	3.25	0	0
44	β-Pinene 	-44.9027	-48.4079	136.23	3.42	0	0

TABLE 2
IN-SILICO PREDICTION OF TOXICITY:

Phytochemicals	Predicted LD_{50}	Predicted Toxicity Class	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Bismurrayaquinone A	296mg/kg	3	Inactive	Inactive	Highly Inactive	Slightly Active	Inactive
Mahanimbinol	2300mg/kg	5	Highly Inactive	Inactive	Highly Inactive	Inactive	Highly Inactive
Mukoenine B	1000mg/kg	4	Inactive	Inactive	Highly Inactive	Inactive	Highly Inactive
Mahanine	4000mg/kg	5	Inactive	Inactive	Active	Inactive	Highly Inactive
Mahanimbicine	4000mg/kg	5	Inactive	Inactive	Active	Inactive	Highly Inactive
Murrayanol	2300mg/kg	5	Inactive	Inactive	Active	Inactive	Highly Inactive
Murrayafoline B	2300mg/kg	5	Inactive	Inactive	Active	Inactive	Highly Inactive
Murrayafoline A	1200mg/kg	3	Inactive	Inactive	Highly Inactive	Active	Highly Inactive
Bicyclomahanimbicine	750mg/kg	4	Inactive	Inactive	Active	Inactive	Highly Inactive
Mahanimbine	4000mg/kg	5	Inactive	Inactive	Active	Inactive	Highly Inactive
Euchrestine B	2300mg/kg	5	Inactive	Inactive	Active	Inactive	Highly Inactive

Koenigine	**522mg/kg**	**4**	**Inactive**	**Inactive**	**Active**	**Slightly Active**	**Highly Inactive**
Murrayazoline	**750mg/kg**	**4**	**Highly Inactive**	**Inactive**	**Active**	**Inactive**	**Highly Inactive**
Koenigicine	**473mg/kg**	**4**	**Inactive**	**Slightly Active**	**Active**	**Slightly Active**	**Highly Inactive**
Mukonicine	**522mg/kg**	**4**	**Inactive**	**Slightly Active**	**Active**	**Slightly Active**	**Highly Inactive**
Koenimbine	**522mg/kg**	**4**	**Inactive**	**Slightly Active**	**Active**	**Slightly Active**	**Highly Inactive**
O-Methylmurrayamine A	**522mg/kg**	**4**	**Inactive**	**Slightly Active**	**Active**	**Slightly Active**	**Highly Inactive**
Mukoline	**640mg/kg**	**3**	**Inactive**	**Inactive**	**Slightly Active**	**Slightly Active**	**Highly Inactive**
Murrayazolinol	**750mg/kg**	**4**	**Inactive**	**Inactive**	**Active**	**Inactive**	**Inactive**
Osthol	**2905mg/kg**	**5**	**Inactive**	**Inactive**	**Inactive**	**Inactive**	**Inactive**
Mukoenine A	**2300mg/kg**	**5**	**Inactive**	**Inactive**	**Highly Inactive**	**Inactive**	**Highly Inactive**
Mukonal	**800mg/kg**	**4**	**Slightly Active**	**Inactive**	**Highly Inactive**	**Slightly Active**	**Highly Inactive**
Murrayacine	**522mg/kg**	**4**	**Inactive**	**Inactive**	**Active**	**Inactive**	**Highly Inactive**
Murrayone	**2905mg/kg**	**5**	**Inactive**	**Inactive**	**Inactive**	**Inactive**	**Inactive**
Phebalosin	**1213mg/kg**	**4**	**Inactive**	**Inactive**	**Highly Inactive**	**Inactive**	**Highly Inactive**

Murrayanine	**1190mg/kg**	**4**	**Slightly Active**	**Inactive**	**Active**	**Highly Inactive**	**Highly Inactive**
Mukolidine	**280mg/kg**	**3**	**Slightly Active**	**Inactive**	**Active**	**Slightly Active**	**Highly Inactive**
Glycozoline	**1530mg/kg**	**4**	**Inactive**	**Inactive**	**Inactive**	**Active**	**Highly Inactive**
Umbelliferone	**10000mg/kg**	**6**	**Inactive**	**Active**	**Highly Inactive**	**Highly Inactive**	**Inactive**
Isolongifolene	**5000mg/kg**	**5**	**Highly Inactive**	**Inactive**	**Highly Inactive**	**Highly Inactive**	**Inactive**
Rutin	**5000mg/kg**	**5**	**Highly Inactive**	**Highly Inactive**	**Active**	**Highly Inactive**	**Inactive**
Myricetin	**159mg/kg**	**3**	**Inactive**	**Slightly Active**	**Highly Inactive**	**Slightly Active**	**Highly Inactive**
Catechin	**10000mg/kg**	**6**	**Highly Inactive**	**Inactive**	**Highly Inactive**	**Inactive**	**Highly Inactive**
Quercetin	**159mg/kg**	**3**	**Inactive**	**Slightly Active**	**Highly Inactive**	**Slightly Active**	**Highly Inactive**
Kaempferol	**3919**	**5**	**Inactive**	**Highly Inactive**	**Highly Inactive**	**Inactive**	**Highly Inactive**
Apigenin	**2500mg/kg**	**5**	**Inactive**	**Inactive**	**Highly Inactive**	**Inactive**	**Highly Inactive**
2-Hydroxy-4-methoxy-3,6-dimethylbenzoic acid	**500mg/kg**	**4**	**Inactive**	**Highly Inactive**	**Highly Inactive**	**Highly Inactive**	**Highly Inactive**
Ascorbic acid	**3367mg/kg**	**5**	**Highly Inactive**	**Highly Inactive**	**Highly Inactive**	**Highly Inactive**	**Inactive**
Icariside B1	**1500mg/kg**	**4**	**Highly Inactive**	**Highly Inactive**	**Active**	**Highly Inactive**	**Highly Inactive**

Squalene	**5000mg/kg**	**5**	**Highly Inactive**	**Highly Inactive**	**Highly Inactive**	**Highly Inactive**	**Highly Inactive**
Epiloliolide	**34mg/kg**	**2**	**Inactive**	**Active**	**Highly Inactive**	**Inactive**	**Highly Inactive**
Loliolide	**34mg/kg**	**2**	**Inactive**	**Slightly Active**	**Highly Inactive**	**Inactive**	**Highly Inactive**
Sabinene	**5000mg/kg**	**5**	**Highly Inactive**	**Inactive**	**Inactive**	**Highly Inactive**	**Highly Inactive**
Beta-Pinene	**4700mg/kg**	**5**	**Highly Inactive**	**Inactive**	**Highly Inactive**	**Highly Inactive**	**Highly Inactive**

DISCUSSION:

Cell membranes containing unsaturated phospholipids and cholesterol undergo peroxidation, which results in the intracellular formation of lipid hydroperoxide by-products such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). These byproducts damage cells, at least partially, by oxidatively altering proteins and causing them to become inactive. Apart from GPx4, various other proteins like GPx1 and glutathione S-transferase type α are engaged in the process of cellular detoxification through the elimination of lipid hydroperoxides. Though GPx4 can directly act on these toxic substances, GPx1 is thought to reduce lipid hydroperoxides after they are first released from the cell membrane and cleaved by phospholipase A2 (PLA2). Lipid hydroperoxide by-products have been connected to a number of illnesses, including UV-induced carcinogenesis, ischemia-reperfusion, and atherogenesis. 4-HNE has been linked to Alzheimer's disease as an oxidative stress metabolite and is linked to inflammation, neurodegenerative diseases, adult respiratory distress syndrome, and atherogenesis. Alzheimer's disease has also been linked to oxidative stress caused by mitochondrial injury, lipid peroxidation, and protein oxidation. An individual with Alzheimer's disease also showed increased levels of 4-hydroxynonenal, a by-product of oxidized lipids, in their brain tissue. These findings imply that GPx4 may play a part in neurodegenerative illnesses by eliminating lipid hydroperoxides[35].

Most common ROS is O_2^-. As a key enzymatic component in the detoxification process of superoxide radicals, SOD is a metalloenzyme that serves as the first line of defense against free radicals. It was discovered that deactivating one SOD allele in human hAPP transgenic mice resulted in a depletion of microtubule-associated protein 2 (neuronal dendritic marker) in the hippocampus and neocortex, decreased astrocytosis, promoted cerebrovascular amyloid gliosis, and plaque-dependent neuritic dystrophy. SOD plays a protective role in neurodegeneration and has been shown to protect the ageing brain against human APP (hAPP)/Aβ-induced impairments.

Regarding behavioral problems, behavior changed due to reduced disinhibition and lower anxiety levels when one SOD allele was absent. Aβ aggregation was accelerated by earlier research on mice lacking SOD in Tg2576 AD. According to other research, mice that overexpressed SOD had fewer memory problems and less plaque development. By efficiently addressing oxidative damage, SOD supplementation was able to mitigate the presentation of cognitive deficits and contrast the noted aggravation of all AD-like characteristics[36].

A neurodegenerative condition called Parkinson's disease (PD) is typified by the death of dopamine-producing neurons in the midbrain. Numerous environmental and genetic factors are thought to be connected to this loss. It has been determined that oxidative stress is one of the elements that causes Parkinson's disease to begin and worsen. Nonetheless, research endeavors persist to validate the correlation and process linked to oxidative stress and Parkinson's disease[37]. A multitude of cellular signaling molecules, such as proteins, enzymes, free radicals, etc., are involved in the intricate process of oxidative stress and can either increase or decrease in response to cellular damage. There are two methods to measure oxidative stress: determining a cell's antioxidant level or using oxidative stress biomarkers. Parkinson's disease primarily affects the neuromelanin-containing dopaminergic neurons of the substantia nigra; hyperoxidation phenomena are thought to play a role in this dopaminergic cell death. The main enzyme that guards against the toxicity of hydrogen peroxide is glutathione peroxidase[38].

One enzyme system linked to the oxidant stress model of Parkinson's disease (PD) pathogenesis is superoxide dismutase (SOD). This study aimed to evaluate SOD activity in red blood cells and whole blood from patients with Parkinson's disease (PD) at both early and advanced stages of the illness. The study included fifteen PD patients in stages I and II (group A), fifteen in stages III and IV (group B), and fifteen normal controls. Both red blood cells and whole blood had their SOD activity measured. SOD activity in red blood cells and whole blood was statistically significantly lower in patients in group B. There was no correlation found between L-Dopa treatment and SOD activity, but a negative correlation was seen between SOD activity and the length of the illness. According to our findings, PD patients gradually experience a defect in SOD activity. Uncertainty surrounds whether this is an epiphenomenon or a later sign of deterioration of the antioxidant mechanism[39].

Although GPX1 plays complex, dichotomous roles as both a tumor suppressor and a promoter in various cancers, it is abnormally elevated in the majority of cancer types. To control the biological behaviors of tumors, such as cell proliferation, apoptosis, invasion, immune response, and chemoresistance, GPX1 can take part in a number of signaling pathways[40].

Low Se levels and plasma GPx activity have been linked to increased lipid peroxidation in cancer patients with metastatic disease, indicating that GPx3 loss contributes to systemic oxidative stress. There have been reports of decreased plasma GPx3 expression in cancer patients with a variety of tumor types, including colorectal and uterine cancers. Hepatocellular carcinoma (HCC), glioblastoma, and non-small-cell lung cancer (NSCLC) patients have lower levels of GPx3 expression in their tumor tissue and plasma when compared to healthy controls. Antioxidant enzymes, as previously discussed, frequently exhibit dichotomous expression and play context-dependent roles in cancer cells, observations that are also made for GPx3[41].

Reduced activity of antioxidant enzymes can lead to oxidative stress and the development of human cancers. SODs, which act as the first line of defense against radicals known as superoxide. Breast cancer patient samples exhibit reduced Cu-ZnSOD activity. Cu-ZnSOD activity was considerably reduced in gastric samples (adenocarcinoma) compared to normal mucosa in both healthy tissues and cancer samples. During the development of cancer, MnSOD is frequently dysregulated. MnSOD expression levels are reduced in human breast cancer patients' fixed tissue samples, which include invasive ductal carcinoma (IDC) and adjacent cancer-free tissues as well as ductal carcinoma in situ (DCIS). High MnSOD expression has been linked to improved disease-specific survival in human oral cancers, particularly in patients with moderate to poor cell differentiation and early-stage buccal mucosal squamous cell carcinomas. Research has indicated that lower MnSOD levels are linked to higher occurrences of esophageal adenocarcinoma (EAC) in human esophageal cancers. The findings demonstrated that when the SOD mimetic was used prior to tumor promoter treatment, tumor incidence was reduced by 40%. It's interesting to note that tumor incidence dropped to 80% when the mimetic was used after tumor promoter treatment[42].

Superoxide dismutase activity was not associated with any risk, but glutathione peroxidase 1 activity was one of the best univariate predictors of the risk of cardiovascular events. Greater glutathione peroxidase 1 activity was inversely correlated with a lower risk of cardiovascular events (P for trend <0.001). Patients with the highest quartile of glutathione peroxidase 1 activity had a higher hazard ratio (0.29) with a 95% confidence interval (0.15-0.58; P<0.001) than those with the lowest quartile. Although sex and smoking status had an impact on glutathione peroxidase 1 activity, this subgroup's predictive ability was not compromised. Following correction for these and additional cardiovascular risk factors, there was almost no change in the inverse relationship between glutathione peroxidase 1 activity and cardiovascular events[43].

In part, by controlling apoptosis, EC-SOD guards against cardiomyocyte death, collagen replacement of tissue, and consequent loss of cardiac function. The loss of cardiac function is facilitated by both of these pathological reactions: remodeling by fibrosis and cellular apoptosis [44]. Reactive oxygen species (ROS) production and reduced antioxidant reserves are linked to left ventricular hypertrophy and dysfunction following myocardial infarction (MI), indicating that oxidative stress may play a role in ventricular remodeling, the emergence of ventricular hypertrophy, and the development of congestive heart failure (CHF). The first defense against ROS is superoxide dismutase (SOD). Three SOD isozymes have been identified: extracellular SOD (EC-SOD), mitochondrial manganese SOD (Mn-SOD), and copper/zinc-containing SOD (CuZn-SOD), which is primarily found in the cytosol. Smooth muscle, endothelial, and fibroblast cells secrete EC-SOD, a glycoprotein that binds to sulfated polysaccharides like heparin and heparan sulfate as well as other matrix components, into the extracellular fluid. Because heparan sulfate is abundant in the extracellular matrix and on the surface of endothelial cells, EC-SOD binds to both of these surfaces. A higher incidence of hypertension and an increased risk of ischemic heart disease are observed in patients whose EC-SOD binding to endothelial cells is decreased as a result of substitution of glycine (R213G) for arginine-213.

This suggests that impaired EC-SOD binding or decreased myocardial EC-SOD content can increase the vulnerability to cardiovascular disease. A number of recent investigations have revealed a decrease in EC-SOD expression in failing hearts, which has been linked to evidence of increased endothelial dysfunction and myocardial oxidative stress. The study's main new findings are that (i) EC-SOD−/− hearts experienced greater hypertrophy and fibrosis after MI compared to wild type hearts, with a greater increase in LV cross section at end systole and a greater decrease in LV ejection fraction; (ii) EC-SOD−/− hearts experienced a greater increase in oxidative stress than wild type hearts, as indicated by nitrotyrosine; and (iii) EC-SOD−/− hearts activated myocardial MAPK signaling pathways in both unstressed and infarcted hearts. To the best of our knowledge, these results represent the first proof that EC-SOD modifies activation of the MAPK signaling pathway and has a protective effect against MI-induced ventricular remodeling[45].

CONCLUSION:

The bioactive molecules of *Murraya koenigii* have a potent antioxidant effect and low level of toxicity that gives valuable insight for optimizing lead molecules to combat free radical generation or oxidation.

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