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Original Article

In silico Investigation on Phytoconstituents in *Pamburus missionis* S. for Antioxidant Activity

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ABSTRACT

Background: *Pamburus missionis* is geographically originated from southern India and it has been used for ailments. **Objectives:** This current research was performed to analyze *in silico* evaluation of phytoconstituents present in *Pamburus missionis* for antioxidant activity. **Materials and Methods:** *In silico* activity of the isolated constituents for antioxidant activity was carried out by Autodock 4.0 and absorption, distribution, metabolism, excretion/toxicity assessed by online tools. **Results:** The results revealed that the phytocompounds, benzoic acid 2,3-dimethyl showed the good docking score of -5.8 kcal/mol, which was a mere docking score of standard curcumin, i.e., -6.6 kcal/mol hence proving that a good binding compatibility among the ligand and the receptor site NADPH oxidase. The Absorption, distribution, metabolism, excretion/toxicity evaluation of phytoconstituents assures that they had obeyed Lipinski's guideline of five suggesting their safety consumption. **Conclusion:** To conclude, *Pamburus missionis* can be a good resource of antioxidant activity and simulation studies is needed to ensure the antioxidant activity of benzoic acid 2,3-dimethyl.

Keywords: Lipinski's, *Pamburus missionis, in silico*, Benzoic acid 2,3-dimethyl, Docking.

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INTRODUCTION

Rutaceae family comprises of about 150 genera and 1310 species out of 71 species were identified only in India. The plants under family were widely spread in tropical and temperate regions. *Pamburus* genus is characterized crown compact or dense. Leaflets will have the fragrance of lemon when crushed. Fruits will be broad and long usually colored of orange to yellowish.[1] All the species under this particular genus of *Pamburus* were categorized under subtribe of Triphasiinae, as it consists of leaves of very short, non-articulated petioles. Earlier investigations were carried out to evaluate on antiarthritic and anti-inflammatory activity.[2-3]

Oxidative stress is the risk factor leads to numerous chronic diseases. The free radicals and other reactive oxygen species are identified to be involved in the pathogenesis of diseases such as asthma, inflammatory diabetes, cancers, atherosclerosis and as many. Reactive oxygen species are said to be cause for the human aging.[4-5] Many antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.[6] Herbal plants are considered as good antioxidant sources since ancient times. Hence the current study is focused on to evaluate *in vitro* and *in silico* antioxidant activity of *Pamburus missionis* Swingle.

Discovery of therapeutic drugs is possible by molecular docking in multiple ways like Identification, screening, designing, prediction and synthesis of chemical compounds. Molecular docking is considered as a efficient method for the designing, synthesis and discovery of therapeutically important drugs. It is being implemented in medicinal chemistry, protein engineering, cheminformatics, bioremediation and many other biological and medicinal fields. Molecular docking method has been used to predict potent drug molecules especially from naturally occurring compounds against various disease. Molecular docking is cost and time effective to analyze complexity of protein-ligand interaction.[7-8]

MATERIALS AND METHODS

Molecular docking: Receptor and Ligand Preparation

In earlier study, the chemical composition of *Pamburus missionis* was investigated by GC and GC-MS.[9] The structures of phytoconstituents in *Pamburus missionis* was retrieved by PubChem and the receptor NADPH oxidase was retrieved from

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the protein data bank (PDB ID: 2CDU) www.rcsb.org/pdb. The water particles were removed from the pdb document and polar hydrogen bonds were included.

To decide the coupling mode and cooperation of the selected compounds and target, docking studies were performed by AutoDock/vina. The pdbqt files of the receptor protein, and *Pamburus missionis compounds alongside the grid box getting at the dynamic site of the receptor for compound interaction was done through Auto Dock. The framework size limits along X, Y, and Z coordinates was scaled to permit appropriate binding adaptability at the docked site. The pdbqt documents were composed into a design (conf) file. The binding investigations were carried out by Discovery Studio (BIOVIA). The physiochemical properties* and pharmacokinetics of the selected compounds were anticipated by openly accessible online SwissADME programming.

RESULTS AND DISCUSSION

In-silico Study of Phytoconstituents in *Pamburus missionis*

To evaluate the possibility of substances being accountable for antioxidant activity, the docking score was investigated to verify the prospective binding affinity. The phytoconstituents were also analyzed to predict their absorption, distribution, metabolism, exertion and toxicity by using online tools. 13 out of 18 phytocompounds strictly obeyed Lipinski's Rule of 5. The phytocompounds obeyed Lipinski's Rule were selected for further study and the remaining phytocompounds were excluded. These phytocompounds are enlisted in Table 1 along with their PubChem ID and the canonical smiles were represented.

Docking studies revealed that out of all phytoconstituents Benzoic acid 2,3 dimethyl had the docking score of -5.8kcal/ mol, which showed conventional hydrogen bonding interactions (PHE429), Van der Waals interaction (ASN434, ASP430, TYR435, VAL398, HIS396, TYR363) and Pi-alkyl bonding (LEU424). No phytocompounds formed hydrophobic interactions. However, most of the phytocompounds shown van der Waals interaction. Among 13 phytoconstituents, Nonanal and Terpinen-4-ol had shown unfavorable Acceptor-acceptor bonding (GLN400, SERI401) and unfavorable Donor-donor bonding (SER401) respectively. Citral had shown no hydrogen bonding, Pi-alkyl and van der Waals interaction rather it shown alkyl bonding (LEU424, VAL404, MET423). 1-Tridecanol had shown no hydrogen bonding and Pi-alkyl bonding however shown van der Waals interaction (GLN400, ASN434, ASP430, TYR435) and Pi-sigma bonding (PHE429). Dodecane had shown no van der Waal and hydrogen bonding interaction however it shown Pi-alkyl interaction (HIS396, LEU424, PHE429). The docking score outcomes were predicted in Table 2 and the interactions between protein and ligand were shown in 2D and 3D (Figure 1 and 2).

A plant comprises of efficient complex enzymatic and non-enzymatic antioxidant defense systems to keep away from the toxic effects of free radicals. These enzymatic systems are Super Oxide Dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), NADPH oxidase and glutathione reductase (GR). The non-enzymatic systems are ascorbic acid, glutathione, proline, carotenoids, terpenoids, phenolic acids, flavonoids, etc.[10-11] As non-enzymatic systems includes the secondary metabolites works against the free radicals, the phytoconstituents from *Pamburus missionis* were choosen for evaluating antioxidant activity to examine whether they suppresses the radicals.

Oxygen consumption is an utmost importance in human body however under some physiological conditions 1-3% of oxygen gets converted into superoxide. Even though they are responsible for various functions in physiological processes like killing of microbes, gene transcription, they show their impact on DNA, proteins or lipids. This impact brings out damage in DNA, proteins or lipids results in development of diseases like CVD, cancer etc., To overcome this damage human body antioxidant defense system, consists of antioxidant enzymes like superoxide dismutase or catalase. Apart from neutraceuticals, polyphenolic

Table 1: ADMET Analysis of phytoconstituents in *Pamburus missionis*.

Ligands	Molecular Formula	Mol. Weight (g/ml)	Drug Likeness	Molar Refractivity	Consensus Log Po/W	H bond Acceptor	H Bond donors
Pinene	$C_{10}H_{16}$	136.23	1	45.22	3.15	0	0
1-Linalol	$C_{10}H_{18}o$	154.25	0	50.44	2.66	1	1
γ-Terpinene	$C_{10}H_{16}$	136.23	0	47.12	3.35	0	0
Nonanal	$C_{9}H_{18}o$	142.24	0	45.58	2.78	1	0
Terpinen-4-ol	$C_{10}H_{18}o$	154.25	0	48.80	2.60	1	1
Nerol	$C_{12}H_{26}$	154.25	1	50.40	2.78	1	1
Dodecane	$C_{10}H_{16}o$	170.33	0	59.80	4.94	0	0
Citral	$C_{10}H_{14}o$	152.23	0	49.44	2.71	1	0
Thymol	$C_{10}H_{14}o$	150.22	0	48.01	2.80	1	1
Eugenol	$C_{10}H_{12}o_2$	164.20	0	49.06	2.25	2	1
10-undecnoic acid methyl ester	$C_{12}H_{22}o_2$	198.30	0	60.61	3.65	2	0
Benzoic acid 2,3-dimethyl	$C_{9}H_{10}O_2$	150.17	0	43.33	2.16	2	1
1-Tridecanol	$C_{13}H_{28}O$	200.36	0	65.77	4.29	1	1
Heptadecane	$C_{17}H_{36}$	240.47	1	83.83	6.79	0	0
1-Hexadecanoic acid	$C_{16}H_{32}o_2$	256.42	1	80.80	5.20	2	1
Nonadecane	$C_{19}H_{40}$	268.52	1	93.45	7.54	0	0
Tetradeconic acid dodecyl ester	$C_{26}H_{52}O_2$	396.69	1	128.38	8.78	2	0
Ricinoleic acid	$C_{18}H_{34}O_3$	298.46	0	91.10	4.82	3	2
Curcumin	$C_{21}H_{20}O_6$	368.4	0	102.80	3.03	6	2

Table 2: Docking score of Phytoconstituents at the active site of receptor NADPH Oxidase.

Ligands	Highest to the Lowest mode of conformation with corresponding RMS binding affinities in ΔG (Kcal/mol)								
	1	2	3	4	5	6	7	8	9
Pinene	−4.6	−4.6	−4.5	−4.4	−4.4	−4.3	−4.1	−4.1	−4.0
1-Linalol	−4.8	−4.2	−4.2	−4.1	−4.1	−4.0	−4.0	−4.0	−4.0
γ-Terpinene	−4.9	−4.8	−4.4	−4.4	−4.3	−4.2	−4.2	−4.2	−4.1
Nonanal	−3.6	−3.3	−3.3	−3.2	−3.2	−3.2	−3.1	−3.1	−3.1
Terpinen-4-ol	−4.8	−4.8	−4.6	−4.5	−4.4	−4.3	−4.3	−4.3	−4.2
Nerol	−4.3	−4.2	−4.1	−4.0	−4.0	−4.0	−4.0	−3.9	−3.8
Dodecane	−3.5	−3.5	−3.4	−3.4	−3.4	−3.3	−3.3	−3.2	−3.2
Citral	−4.2	−4.1	−4.0	−4.0	−3.8	−3.8	−3.8	−3.8	−3.7
Thymol	−5.1	−5.0	−4.9	−4.7	−4.6	−4.5	−4.4	−4.3	−4.3
Eugenol	−5.1	−5.1	−5.1	−5.0	−5.0	−4.9	−4.6	−4.5	−4.4
10-undecnoic acid methyl ester	−4.0	−3.9	−3.9	−3.9	−3.8	−3.7	−3.7	−3.5	−3.5
Benzoic acid 2,3-dimethyl	−5.8	−5.4	−5.1	−4.8	−4.7	−4.7	−4.6	−4.5	−4.3
1-Tridecanol	−3.6	−3.4	−3.3	−3.3	−3.1	−3.1	−3.1	−3.1	−3.1
Heptadecane	−4.8	−4.8	−3.8	−3.7	−3.1	−3.1	−3.1	−3.0	−3.0
1-Hexadecanoic acid	−4.3	−4.1	−4.0	−4.0	−3.9	−3.9	−3.8	−3.7	−3.7
Nonadecane	−4.2	−4.1	−3.9	−3.8	−3.7	−3.7	−3.5	−3.5	−3.5
Tetradeconic acid dodecyl ester	−5.0	−4.8	−4.8	−4.8	−4.7	−4.7	−4.6	−4.6	−4.5
Ricinoleic acid	−4.7	−4.4	−4.3	−4.2	−4.2	−4.1	−4.1	−4.0	−3.9
Curcumin	−6.6	−6.4	−6.4	−6.3	−6.2	−6.1	−6.1	−6.0	−6.0

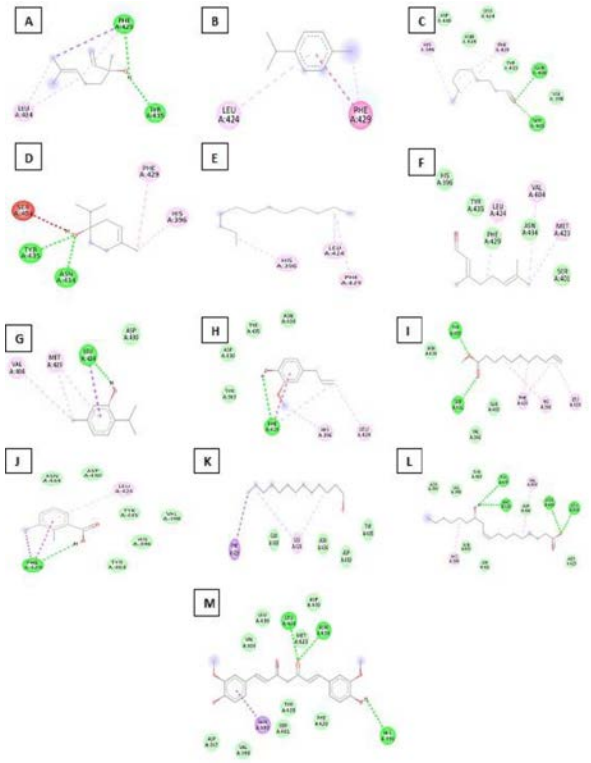

Figure 1: Two dimensional interactions of ligands with receptor NADPH Oxidase.
A) 1-linalol B) γ-terpinene C) Nonanal D) Terpinen-4-ol E) Dodecane F) Citral G) Thymol H)Eugenol I) 10-Undecnoic acid methyl ester J) Benzoic acid 2,3-dimethyl K) 1-Tridecanol L) Ricinoleic acid M) Curcumin.

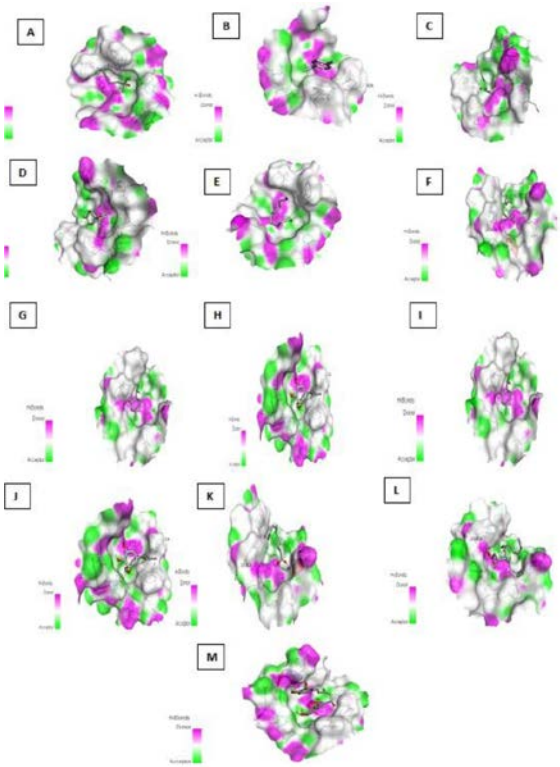

Figure 2: 3-dimensional interactions of ligands with receptor NADPH Oxidase.
A) 1-linalol B) γ-terpinene C) Nonanal D) Terpinen-4-ol E) Dodecane F) Citral G) Thymol H)Eugenol I) 10-Undecnoic acid methyl ester J) Benzoic acid 2,3-dimethyl K) 1-Tridecanol L) Ricinoleic acid M) Curcumin.

Table 3: GI absorption of phytoconstituents in *Pamburus missionis*.

Ligands	PubChem ID	Canonical Smiles	GI Absorption
Pinene	15837102	CC1=C2CC(C2(C)C)CC1	Low
1-Linalol	6549	CC(=CCCC(C)(C=C)O)C	High
γ-Terpinene	7461	CC1=CCC(=CC1)C(C)C	Low
Nonanal	31289	CCCCCCCCC=O	High
Terpinen-4-ol	11230	CC1=CCC(CC1)(C(C)C)O	High
Nerol	643820	CC(=CCCC(=CCO)C)C	High
Dodecane	8182	CCCCCCCCCCCC	Low
Citral	638011	CC(=CCCC(=CC=O)C)C	High
Thymol	6989	CC1=CC(=C(C=C1)C(C)C)O	High
Eugenol	3314	COC1=C(C=CC(=C1)CC=C)O	High
10-undecnoic acid methyl ester	8138	COC(=O)CCCCCCCCC=C	High
Benzoic acid 2,3-dimethyl	11782	CC1=C(C(=CC=C1)C(=O)O)C	High
1-Tridecanol	8207	CCCCCCCCCCCCCO	High
Heptadecane	12398	CCCCCCCCCCCCCCCCC	Low
1-Hexadecanoic acid	985	CCCCCCCCCCCCCCCC(=O)O	High
Nonadecane	12401	CCCCCCCCCCCCCCCCCCC	Low
Tetradeconic acid dodecyl ester	74881	CCCCCCCCCCCCCC(=O)OCCCCCCCCCCCC	Low
Ricinoleic acid	643684	CCCCCCC(CC=CCCCCCCCC(=O)O)O	High
Curcumin	969516	COC1=C(C=CC(=C1)C=CC(=O)CC(=O)C=CC2=CC(=C(C=C2)O)OC)O	High

compounds and terpenoids have gained great interest these days.[12] Hence the plant *Pamburus missionis* predominantly having the presence of terpenoids and phenolic compounds which acts as free radicals defense system.

Earlier investigations on *Pamburus missionis* were examined for antimicrobial activity on 11 different bacterial species and 8 fungal species and also analyzed for phytoconstituents Spiroheptadiene, Hentriacontane and 13- Docosenamide etc by GC-MS analysis.[13-14] The phytoconstituents were earlier reported by modern chromatographical analysis.

Numerous biologically active compounds were reported from these plants such as phenolic compounds, alkaloids, flavonoids and terpenoids. Investigation on *Thuidium tamariscellum* for antioxidant potentialities in terms of DPPH, ABTS, H_2O_2, FRAP and metal chelating ability and it is also focused on phytochemical analysis by FTIR. The exhibiting of antioxidant activity by thuidium tamariscellum is due to lead components of terpenoids. This work is made evidenced, the prominent role of terpenoids against free radicals.

The docking research disclosed an engrossing perspective between the stated ligands and receptor NADPH oxidase. Protein-ligand interaction showed that 1- linalool, Nonanal, Terpinen-4-ol, Nerol, Thymol, Eugenol, 10-Undecnoic acid methyl ester, Benozoic acid 2,3-dimethyl created hydrogen bonds to TYR 435, PHE 429, SER401, ASN434, LEU424 and GLN400 respectively. The docking score of Benzoic acid 2,3-dimethyl, Eugenol, Thymol and Tetradeconic acid dodecyl ester is around –5.8, –5.1, –5.1 and –5.0 respectively.

In the present study, twelve ligands of *Pamburus missionis* at the receptor NADPH oxidase energetic site and the docking score was computed, making use of Autodock 4.0. The findings revealed that a good docking score of about -5.8 kcal/mol between Benzoic acid 2,3-dimethyl and receptor NADPH oxidase which proved that it strongly suppresses the free radicals, as it shows strong communication with the receptor. It is apprehended that ADMET evaluation for 18 phytoconstituents, made to exclude the phytoconstituents pinene, nerol, Heptadecane, 1-Hexadecanoic acid, Nonadecane and Tetradeconoic acid dedecyl ester as they were not obeyed Lipinski's Rule of 5. The pharmacokinetic study was explored by SwisADME tool revealed γ-terpene, Nonanal, Terpinen-4-ol, Nerol, Citral, thymol, eugenol, 10-undecnoic acid methyl ester, Benzoic acid 2,3-dimethyl, 1-tridecanol, 1- hexadecanoic acid and ricinoleic acid GI absorption is high and the remaining phytoconstituents were shown low depicted in Table 3. Hence these phytoconstituents may be used for antioxidant activity and the herbal formulation may consider to develop by making use of the above phytoconstituents, as their GI absorption is reported as high.

CONCLUSION

Free radicals leads to various diseases. Numerous plant originated phytoconstituents possess antioxidant activity which is not allowed to prone diseases like cancer. The earlier studies on *Pamburus missionis* reveals the presence of terpenoids rich. This paved to screen the antioxidant property of phytoconstituents of *Pamburus missionis* using molecular docking. Further ADMET evaluation is carried on. The molecular docking results had shown the verdict that benzoic acid 2,3-dimethyl revealed the good docking score. A meticulous SAR model is needed to guarantee its bioefficacy. Finally, the outcomes confirm the ethnomedicinal use of *Pamburus missionis* might be a possible resource for scavenging the free radicals.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ADMET: Absorption, Distribution, Metabolism, Excretion, and Toxicityin; **GI:** Gastro Intestinal; **SOD:** Super Oxide Dismutase; **CAT:** catalase; **GPx:** glutathione peroxidase; **GR:** glutathione reductase; **CVD:** CardioVascularDiseases.

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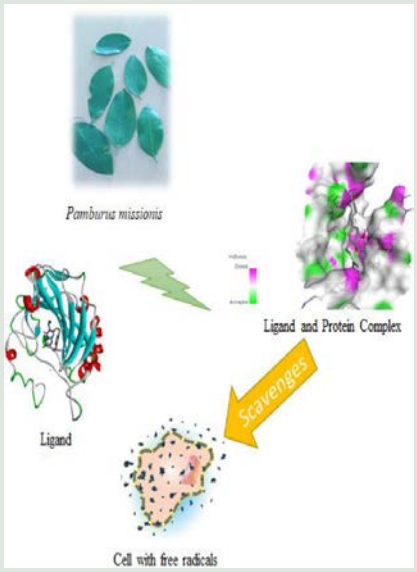
GRAPHICAL ABSTRACT



SUMMARY

- Rutaceae family is predominantly consists of terpenoids leads to selection of plant *Pamburus missionis*.
- Oxidative stress is the risk factor which leads to numerous chronic diseases.
- Molecular docking helps to assess screen, design and gives the prediction of pharmacological action hence used to discovery of lead molecules are high possible.
- Various phytoconstituents present in *Pamburus missionis* is assessed for antioxidant activity by molecular docking studies revealed benzoic acid 2,3-dimethyl scavenges the free radicals.

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