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Molecular docking of embelin against human mono amine oxidase- a (MAO-A) enzyme

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Abstract
Vitiligo is a dermatological disorder. Free radical and reactive oxygen species are well known key factor for the pathogenesis of vitilgo. The present paper reports the formulation of poly herbal capsule, Molecular docking of embelin, a major constitution from the poly herbal capsule with the receptor mono amino oxidase-A enzyme and the *in-vitro* antioxidant activity of the poly herbal capsule by DPPH radical scavenging and total antioxidant assay method. The result showed the inhibitory effect of embelin against the receptor MAO-A. The *in-vitro* antioxidant activity of the formulated poly herbal capsule showed remarkable effect similar to the standard drug Ascorbic acid. The result obtained validates the traditional uses of the formulated poly herbal capsule in the treatment of vitiligo in siddha system of medicine.

Keywords: Embelin, mono amino oxidase- a, vitiligo, poly herbal capsule, insilico investigation, *in-vitro* antioxidant activity

Introduction
Vitiligo is a skin disorder and its pathogenisis is due to excess production of reactive oxygen species. There are various sources to produce reactive oxygen species. Mono amine oxidase- A level is also one of the sources for contributing reactive oxygen species production. In epidermis of patients with vitiligo was found to be increased MAO-A enzyme activity. MAO-A activity increases due to the Norepinephrine synthesis instead of melanin synthesis. MAO-A metabolize the Norephinephrine which results in the production of ROS like Hydrogen peroxide and ammonia which are toxic to melanocytes.
Embelin, a cheif constituent reported in the herb of *Embelia ribes*, which is one of the ingredients in the formulated poly herbal capsule. In siddha system of medicine *Emblica ribes* is traditionally being used for the treatment of vitiligo. Hence to validate the pharmacological activity of embelin, it is planned to formulate the antivitiligo capsule, to investigate by computational screening for the chief constituents present in the formulation and to screen the antioxidant potential of formulated poly herbal capsule.

Materials and Methods
Plant Materials
Following six herbs traditionally claimed for the treatment of vitiligo were choosen such as *Smilex chinensis* (Tuberous root), *Embelia ribes* (Dried fruit), *Withania somnifera* (Root), *Elettaria cardomomum* (Dried fruit), *Psoralea corylifolia* (Dried seed), *Piper longum* (Dried fruit). The above six herbs were botanically identified[4] and authenticated[5] at Plant Anatomy Research Institute, Thambaram Chennai, India (Herbarium No: PARC/2013/105-111).

Preparation of Poly Herbal Formulation
Poly herbal formulation is done by adding 100 mg of tuberous root of *Smilex chinensis*, 50mg of dried fruit of *Embelia ribes*, 100mg of dried root of *Withania somnifera*, 10mg of dried fruit of *Elletaria cardamomum*, 50mg of dried seeds of *Psoralea corylifolia* and 50mg of dried fruit of *Piper longum*. The above ingredients mixed together and pass the poly herbal mixer through sieve number 44# to prepare homogenous blend and filled in the empty gelatine capsule.

Docking study of Embelin against MAO-A Enzyme
To investigate the potential binding mode of inhibitor, the compound Embelin is subjected to molecular docking using the Auto Dock 1.5.4 docking program. The X-ray crystal structure of MAO- A in complex with Harmine was downloaded from the protein data bank (PDBID: 2Z5X) and was used for the docking study. Ligand 2D structures were drawn using Chem Draw Ultra 7.0 (Chem Office 2002). Chem3D Ultra 7.0 was used to convert 2D structure into 3D and the energy minimized using semi-empirical AM1 method.

Minimize energy to minimum RMS gradient of 0.100 was set in each iteration. The structure was saved as PDBQT file format for input to Auto Dock Tools (ADT) version 1.5.4. The ligand structures were then saved in PDBQT file format, for input into Auto Dock version 1.5.4.

For the molecular docking study, protein structure was obtained from the Protein Data Bank; the MAO-A structure PDB ID was 2Z5X. The co crystallized ligand (Harmine) in the MAO-A structure was removed. For the protein structure, all hydrogen atoms were added, lower occupancy residue structures were deleted, and any incomplete side chains were replaced using the ADT version 1.5.4. Further ADT was used to remove crystal water, added Gagteiger charges to each atom, and merged the non-polar hydrogen atoms to the protein structure. The distance between donor and acceptor atoms that form a hydrogen bond was defined as 1.9 Å with a tolerance of 0.5 Å, and the acceptor-hydrogen-donor angle was not less than 120°. The structures were then saved in PDBQT file format, for input into Auto Dock version 1.5.4. A grid box with dimension of 40×40×40 Å and was centered on 41.199, 26.18, -15.419 was created around the binding site of harmine on MAO-A protein using Auto dock tools.

The centre of the box was set at harmine and grid energy calculations were carried out. For the Auto Dock docking calculation, default parameters were used and 50 docked conformations were generated for each compound. In order to verify reproducibility of the docking calculations, the bound ligand (harmine) was extracted from the complexes and submitted for one-ligand run calculation. This reproduced top scoring conformations of 10 falling within root-mean-square deviation (RMSD) values of 0.696 to 1.064 Å from bound X-ray conformation for MAO-A suggesting this method is valid enough to be used for docking studies of other compounds. The Estimated Free Energy of Binding = -6.99 kcal/mol. The outputs were exported to VMD and Pymol for visual inspection of the binding modes and interactions of the compounds with amino acid residues in the active sites.

In vitro antioxidant activity of the formulated poly herbal capsule

Chemicals 95% ethanol, DMSO, Diphenyl-1-picryl hydrazyl, Methanol, Sodium phosphate, ammonium molybdate and sulphuric acid were procured from Sigma Aldrich.

Preparation of test and standard solution

The Ethanolic extract of formulated poly herbal capsule (Hot percolation method by soxhlet apparatus) were dissolved in dimethyl sulfoxide (DMSO) separately and used for *in-vitro* antioxidant assay.

The Ethanolic extract of formulated poly herbal capsule and ascorbic acid, 1000µg/ml were prepared as a final concentration and the absorbance were measured against blank solution that contains the test and standard solution without the reagents. The IC_{50} value of the test and standard were calculated (Uma Bhandari *et al*. 2007).

Diphenyl-1-picryl hydrazyl (DPPH) Radical scavenging method

The extract or standard solution (10µg/ml) was added to DPPH in methanolic solution (100 µM, 200 µl) in a 96-well microtiter plate (Tarsons, Kolata, India). After incubation at 37 ºC for 30 min the absorbance of each solution was determined at 490nm.

Total antioxidant activity

The Total Antioxidant capacity of the test solution was determined with Phospho molydenum using ascorbic acid as the standard. An aliquot of 0.1 ml of test solutions (10, 50, 100, 500, 100µg) solution was combined with 2.0 ml of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95 ºC for 90 min. After the sample had cooled to room temperature, the absorbance was measured at 695 nm against blank in UV spectrophotometer. Increased absorbance of the reaction mixture indicated increased in antioxidant capacity.

Calculation of percentage inhibition

The absorbance of standard and test solution is taken as triplicates and the percentage inhibition of average absorbance reading is calculated as follows

$$\text{Percentage of inhibition} = \frac{\text{Control-Test /Standard absorbance}}{\text{Control absorbance}} \text{ X } 100$$

Results and Discussion

Poly herbal capsules were formulated for the herbs traditionally used for the treatment of vitiligo in the siddha system of medicine (Figure. 1 & Table. 1).

Oxidative stress is one of the key factors for the loss of melanocytes that result in the pathogenesis of vitiligo. The elevated level of Mono amino oxidase-A due to increased reactive oxygen species is observed in the patients having vitiligo. Hence the molecular docking of the chief constituent Embelin present in the anti-vitiligo formulation is done against the receptor Mono amino oxidase-A. It is observed that Embelin has inhibitory effect on MAO-A and the binding energy is calculated as -3.31 as least binding energy and -3.36 as highest binding energy between the ligand and the receptor (Table. 2 & Figure. 2).

The antioxidant activity of poly herbal capsule is screened by two different methods DPPH radical scavenging method and total antioxidant activity determination method. The test drug is compared with the standared drug ascorbic acid and the IC_{50} value is found to be 11.54 µg/ml for the test drug and 8.12 µg/ml for ascorbic acid for DPPH radical scavenging method and 609.15 for the test drug and 273.5 µg/ml for ascorbic acid for total antioxidant activity. The result showed the formulated poly herbal capsule has promising anti-oxidant potential similar to the standard drug ascorbic acid (Table.3-6, Graph. 1-4).

Conclusion

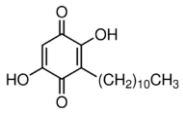
The present paper focuses on the formulation, molecular docking study and screening the antioxidant potential of the poly herbal capsule in the treatment of vitigo. From the research findings it is concluded that one of the chief constituent Embelin present in one of the ingredient *Embelia ribes* in the formulated capsule exhibits inhibitory effect on the receptor MOA-A, a hallmark in the pathogenesis of vitiligo. The ethanolic extract of the ingredients in the formulated poly herbal capsule had remarkable anti-oxidant activity similar to the standard drug Ascorbic acid. The research findings scientifically validate the traditional claim of the ingredients used in the formulation in the siddha system of medicine.

Fig 1: Formulated Poly herbal capsule

Table 1: Formulation profile for the poly herbal capsule

S. No	Siddha Name	Botanical Nomenclature	Part	Quantity
1	Parangi pattai	*Smilex chinensis*	Dried Root	100mg
2	Vai vidangam	*Embelia ribes*	Dried Fruit	50mg
3	Amukkara	*Withania somnifera*	Dried Root	100mg
4	Elarsi	*Elettaria cardamomum*	Dried Fruit	10mg
5	Karboga arsi	*Psoralea corilifolia*	Dried Seed	50mg
6	Thippili	*Piper longum*	Dried Fruit	50mg

Table 2: Molecular Docking of MAO-A with Embelin

S. No	Compound/Ligand	Receptor	Binding energy
1	Embelin 	Mono Amino Oxidase-A 	-3.31 - least binding energy -3.6 – highest binding energy

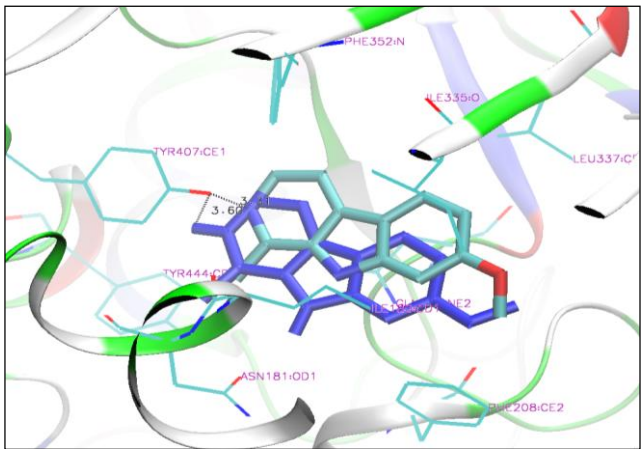


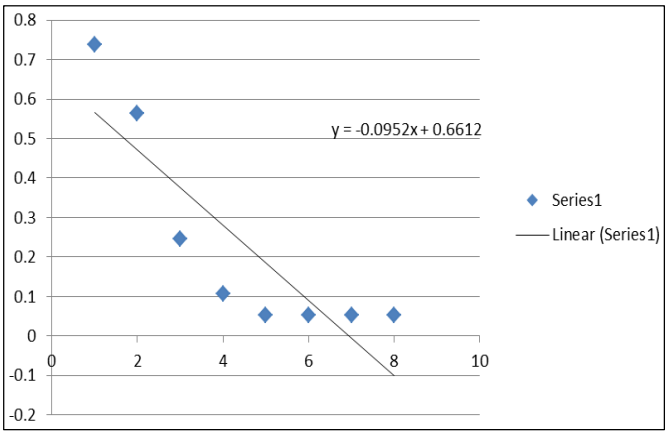
Fig 2: Molecular Docking Of Embelin with Human Mono Amino Oxidase (Mao-A) Enzyme

Table 3: Antioxidant Activity of Ethanolic Extract of Formulated Poly Herbal Capsule by DPPH Scavenging Method

Concentration (µg/Ml)	Absorbance of Test	% Inhibition of Test (µg/Ml)
5 µg	0.738	33.03
10µg	0.564	48.82
50µg	0.246	77.67
100µg	0.106	90.38
200µg	0.052	95.28
500µg	0.052	95.28
1000µg	0.052	95.28
2000µg	0.052	95.28
Control	1.102	

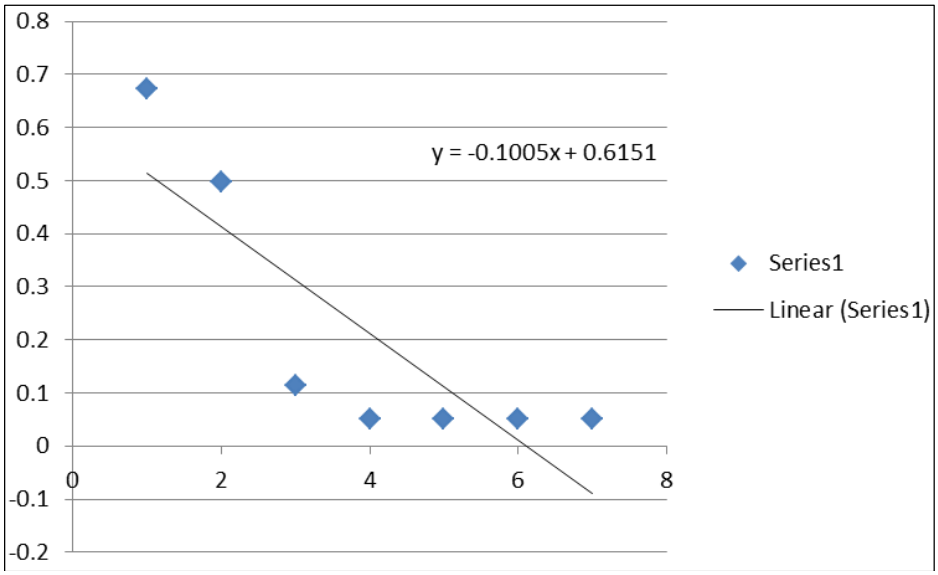
Table 4: Antioxidant Activity of Ethanolic Ascorbic Acid by DPPH Scavenging Method

Concentration (µg/ml)	Absorbance of Standard	% Inhibition of Standard (µg/ml)
5 µg	0.672	39.02
10µg	0.498	54.81
50µg	0.114	89.65
100µg	0.052	95.28
200µg	0.052	95.28
500µg	0.052	95.28
1000µg	0.052	95.28
Control	1.102	



IC_{50} Value: 11.54 µg/ml

Graph 1: Graphical Representation of Antioxidant Activity for Ethanolic extract of formulated poly herbal capsule by DPPH Radical Scavenging Method



IC_{50} Value: 8.12 µg/ml

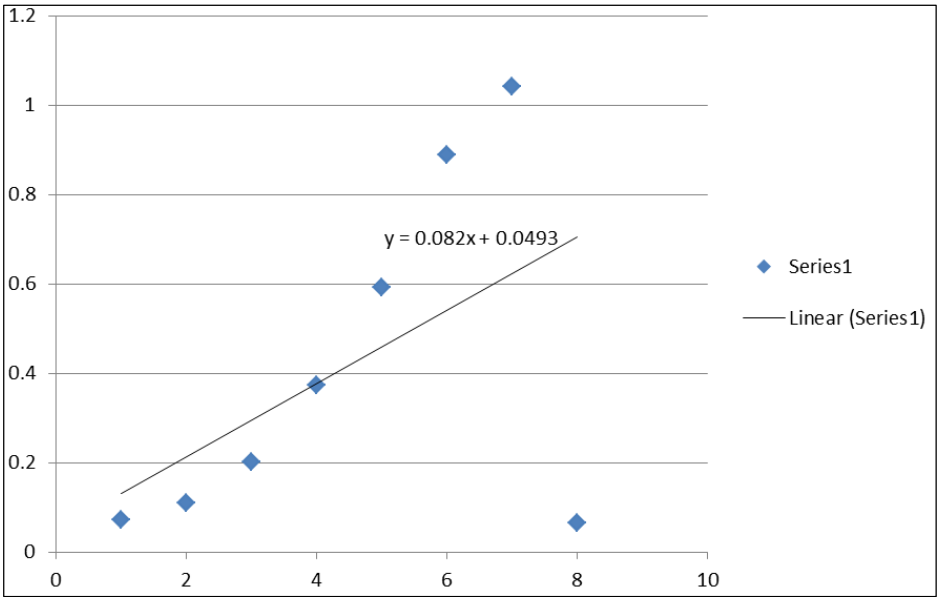
Graph 2: Graphical Representation of Antioxidant Activity for Ascorbic acid by DPPH Radical Scavenging Method

Table 5: Antioxidant Activity of Ethanolic Extract of Formulated Poly Herbal Capsule by Total Antioxidant Assay Method

Concentration (µg/ml)	Absorbance of Test Å
5 µg	0.073
10µg	0.109
50µg	0.202
100µg	0.374
200µg	0.592
500µg	0.888
1000µg	1.042
Control	0.065

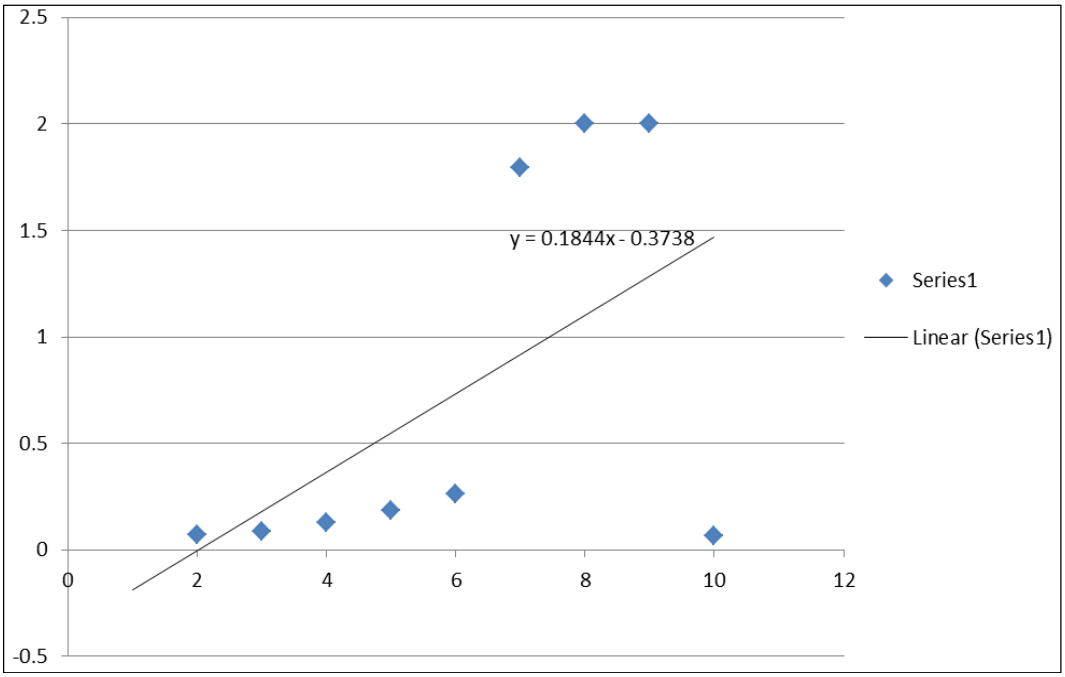
Table 6: Antioxidant Activity of Ascorbic acid by Total Antioxidant Assay Method

Concentration (µg/ml)	Absorbance of Standard Å
0.5µg	0.072
1µg	0.087
3µg	0.127
5µg	0.184
10µg	0.265
50µg	1.794
100µg	1.999
200µg	1.999



IC_{50} Value: 609.15 µg/ml

Graph 3: Graphical Representation of Antioxidant Activity for formulated poly herbal capsule by total antioxidant activity



IC_{50} Value: 273.17 µg/ml

Graph 3: Graphical Representation of Antioxidant Activity for Ascorbic acid by total antioxidant activity

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