

# Comparative analysis and *in-silico* studies of *Oecophylla smaragdina* collected from the states of Chhattisgarh and Maharashtra, India

Sunita Singh\*\*, Kuhi Rai, Arpita Gupte, Mala Parab and Pramodkumar P. Gupta

School of Biotechnology and Bioinformatics, D. Y. Patil Deemed to be University, Navi Mumbai, India.

\*\*sunita.singh@dypatil.edu, dr.sunitads@gmail.com

## Abstract:

The consumption of red weaver ant and their eggs (*O. smaragdina*) have claimed a wide medicinal properties to treat a variety of ailments like cold, whooping cough, high fever, malarial fever, ear pain, etc by the tribal groups across the country. The goal of the study was to analyze the biochemical, antimicrobial, anti-inflammatory and cytotoxicity effects of the consumption of the red weaver ant and their eggs and whether they are fit to consume by the humans imparting the potential to the treatment of claimed diseases. Two samples of the red weaver ants, viz. Bastar (Chhattisgarh) and Gadchiroli (Maharashtra) viz. Bastar (Chhattisgarh) and Gadchiroli (Maharashtra) were compared. The sample were dried, grinded and Soxhlet based extraction was carried out in the methanol solvent respectively. Crude proteins and elements (Zinc and Calcium), Total phenolic content and DPPH radical scavenging was carried out. The antimicrobial potential of the extract was tested against *E.coli*, *S. aureus*, *K. pneumoniae* and *S. pyogenes*. Nitric oxide assay and RBC membrane stabilization test was performed to observe the inhibition of the inflammatory actions. MTT assay was performed for the analysis of cytotoxicity in the sample using PBMNCs cell culture. To deeply understand the pharmacological process, *in-silico* based modelling and analysis was performed. The proximate analysis revealed a significant presence of Zinc in both the samples. The DPPH assay revealed former to have higher % inhibition and lower antioxidant activity i.e., 54.35 mg/AAE ml whereas later sample showed lower % inhibition and higher antioxidant activity i.e., 86.9 mg/AAE ml. The total phenolic contents (Catechol equivalents, mg/g) in the samples were calculated to be 3.9853 and 5.228 mg Catechol eq/ml, respectively. In all the modelled protein 3D structures a maximum number of residues were identified within the allowed regions of Ramachandran Plot, followed by energy minimization in Gromacs. The structures are reported with a good degree of stability according to the potential energy of the protein. The outcome from the protein-protein docking studies reported a strong affinity between both the proteins, supported by numerous hydrogen bonds formation between them. From the *in-silico* analysis, here we predict the plausible interaction between the proteins from the red weaver ants that interacts to the proteins known as drug target from the selected pathogen and hence may be able to inhibit their pathogenicity too in future.

## Background

Many tribal groups eat me & my eggs and call it 'Cheeti chutney'. They say I can cure them.



Wow! But How?



### What We Knew

'Nutritional analysis, volatile composition, antimicrobial and antioxidant properties of Australian green ants (*O. smaragdina*), 2021' by Shanmugam Alagappan et al.

- Determined the nutritional value, volatile compounds, antimicrobial and antioxidant potential in different parts of the body of ant.
- Proximate analysis revealed the anterior part have higher protein fibre content.
- The antimicrobial activity was observed for whole ants and anterior part methanol extracted against *S. aureus*.
- Whole ants methanol and water extracts exhibited higher free radical scavenging and total phenolic content.

### The Research Gap

- Claims by tribal, needs scientific validation to prove the potential for the treatment of dengue, malaria, jaundice, rheumatism, cough and cold and various other gastrointestinal infections.
- Comparative analysis between the species from different geographical areas and ethno-entomological practices.
- Isolation and characterization of silk fibroin protein which is possibly responsible for the synthesis of thread like structure by the ants for weaving of the nests on the tree.
- In-silico*, genomic and computational analysis of proteins and metabolites secreted by red weaver ants.

### The Objectives

- Collection of *O. smaragdina* from the forested areas of Bastar, Chhattisgarh and Gadchiroli, Maharashtra.
- Comparative analysis of biochemical and antimicrobial properties against microbes.
- To determine the elements (Crude protein, Zinc and calcium), Total phenolic content and DPPH activity.
- Determination of Anti-inflammatory properties and Cytotoxicity properties
- In-silico* analysis and Protein docking studies of the proteins and genes found ant.

## Methodology

A. Collection of *O. smaragdina* from the forested areas of Bastar, Chhattisgarh and Gadchiroli, Maharashtra



Sample 1 – Bastar  
Sample 2- Gadchiroli

B. Drying the ants, eggs and grinding in motor and pestle



C. Soxhlet based extraction in methanol solvent



BIOCHEMICAL CHARACTERIZATION

ANTIMICROBIAL ANALYSIS

ANTI-INFLAMMATORY ANALYSIS

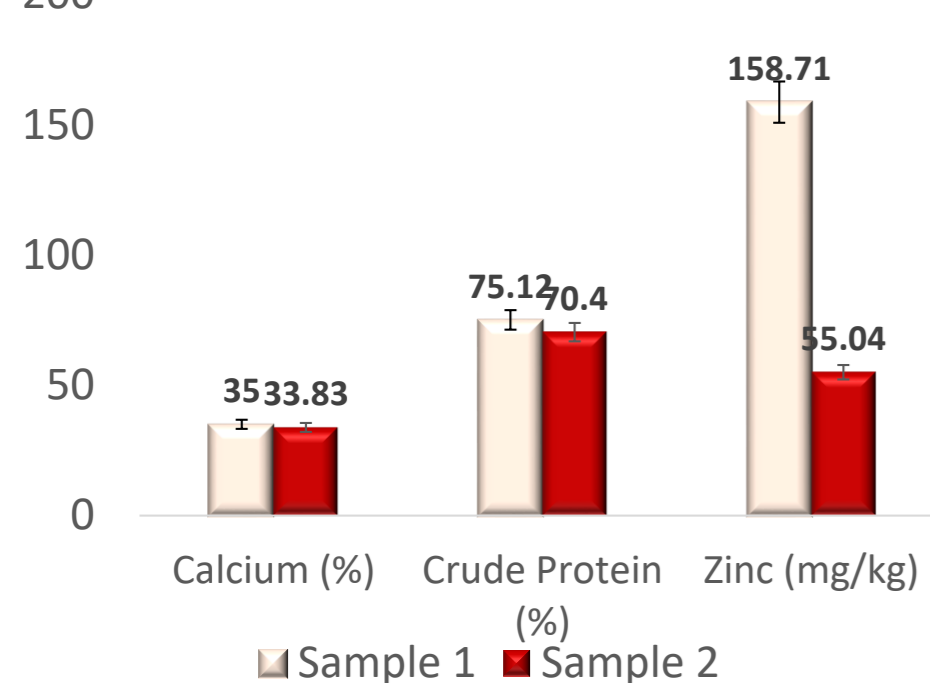
CYTOTOXICITY ANALYSIS

INSILICO ANALYSIS AND MOLECULAR DOCKING

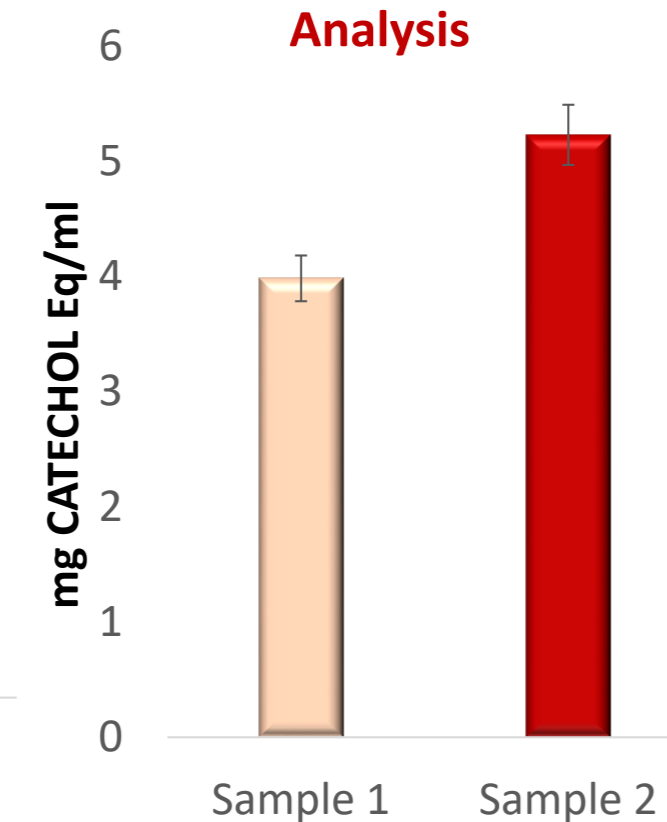
The genes and proteins were retrieved from the NCBI database, protein 3D structure were modelled using SWISS-model server, and the modelled 3D structures were assessed for their ProSA and Ramchandran Plot analysis. The 3D structure optimized using GROMACS package and P-P docking was carried out with the drug target of the gastrointestinal respiratory, Malarial, Dengue, Jaundice and COVID-19 (SARS CoV-2) infectious pathogens using the computational tool, Hex 8.0.0.

## Results

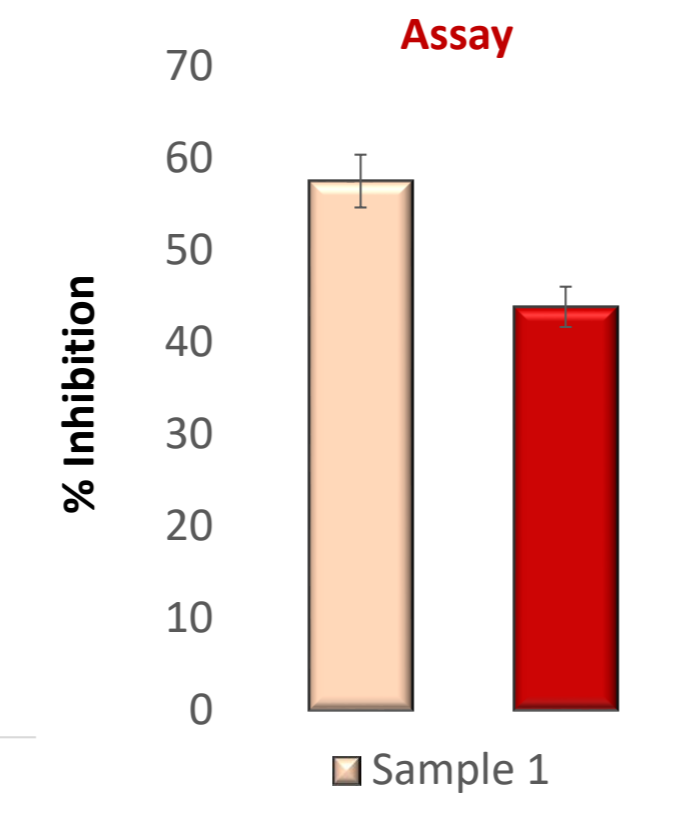
### Proximate Analysis



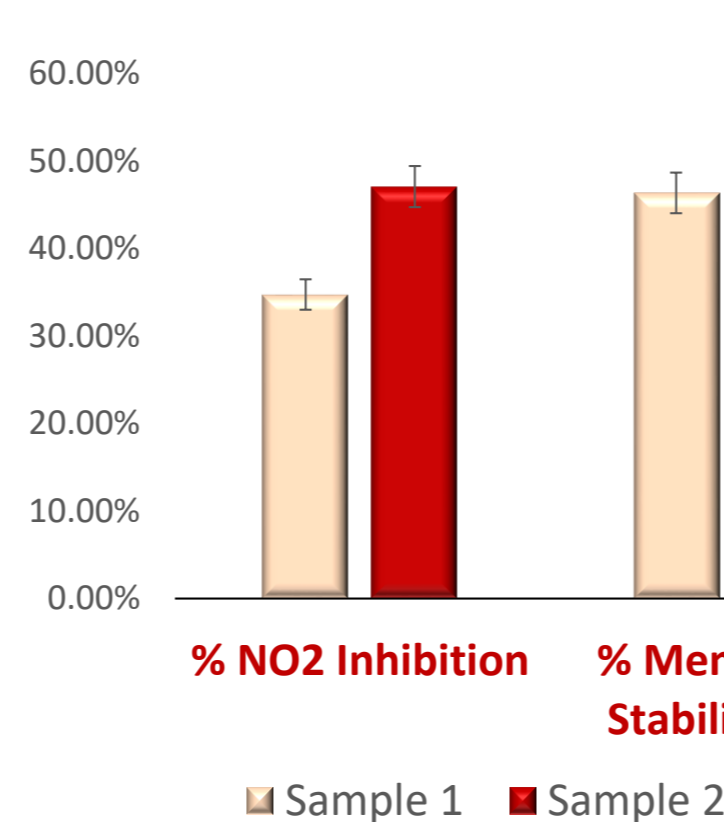
### Total Phenol content Analysis



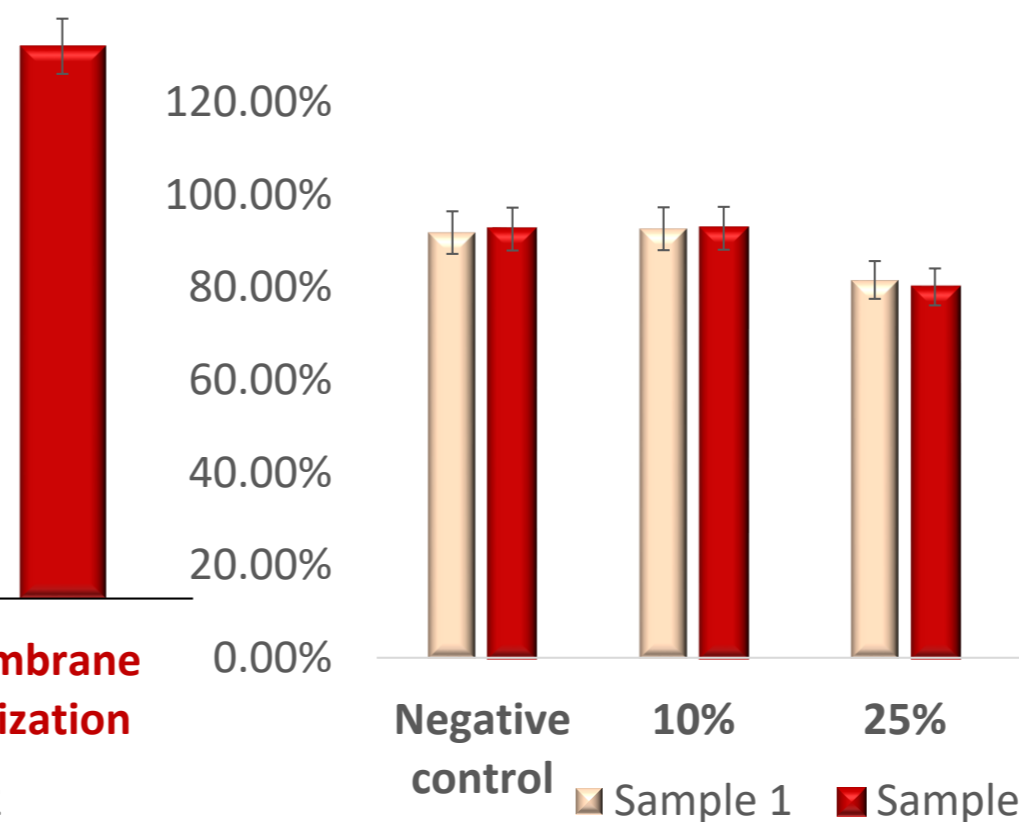
### DPPH Radical scavenging Assay



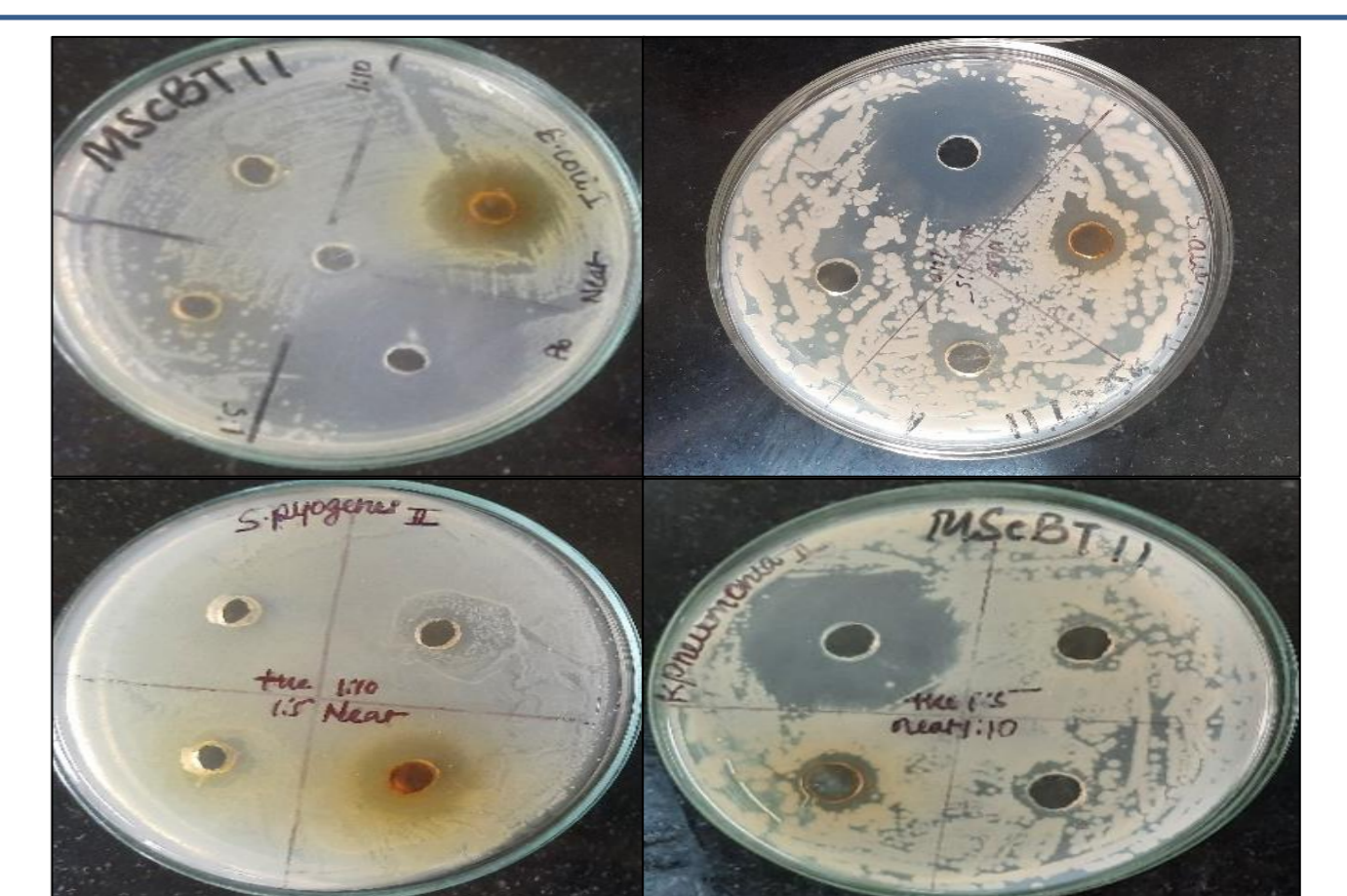
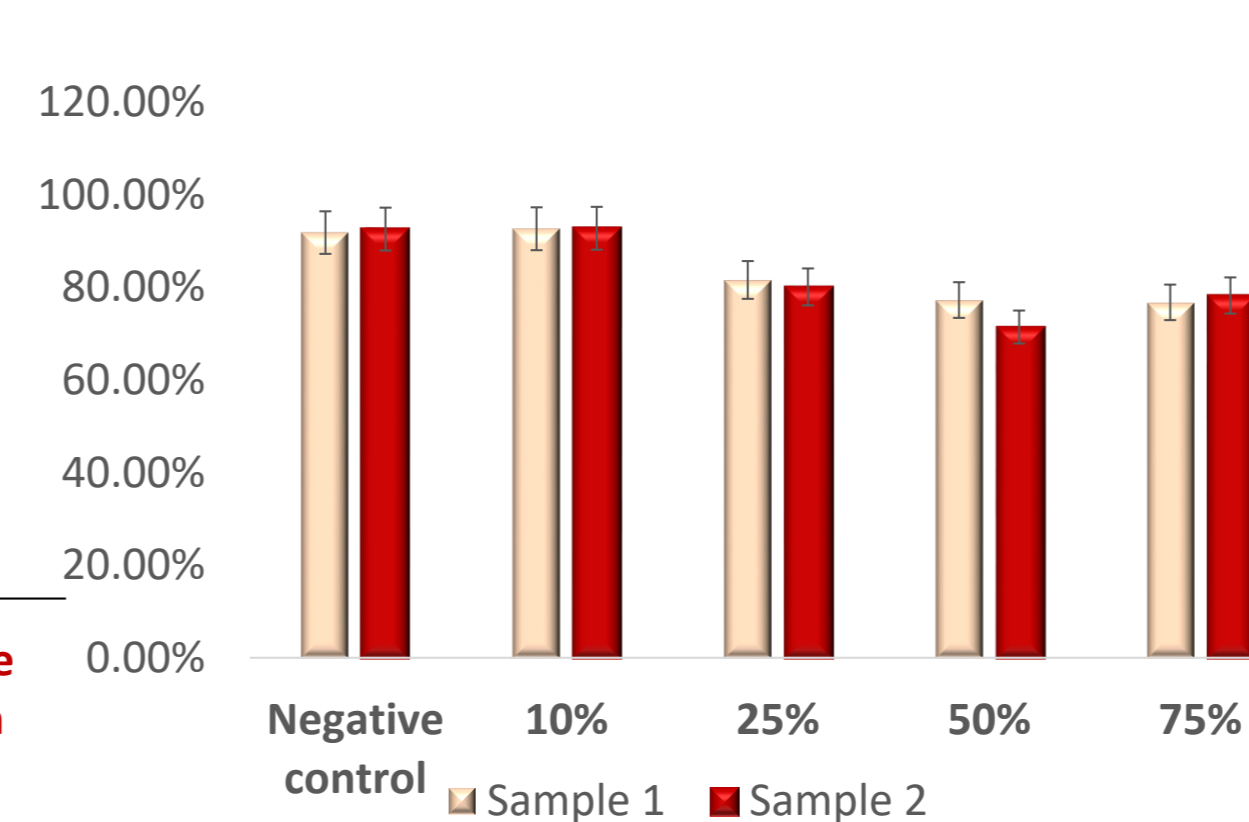
### % NO2 Inhibition



### % Membrane Stabilization



### MTT Assay % Survival



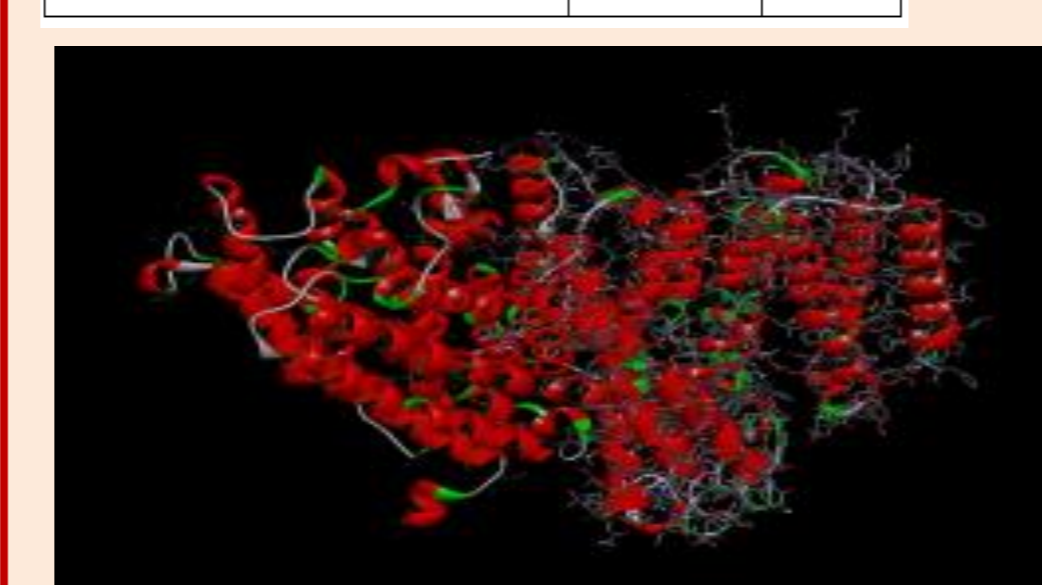
Antimicrobial activity of the methanolic extract against *E. coli*, *S. aureus*, *S. pyogenes* and *K. pneumoniae*

- The proximate analysis revealed a significant presence of Zinc in both the samples.
- The DPPH assay revealed former to have higher % inhibition and lower antioxidant activity i.e., 54.35 mg/AAE ml whereas Sample 2 showed lower % inhibition & higher antioxidant activity i.e., 86.9 mg/AAE ml.
- The total phenolic contents (Catechol equivalents, mg/g) in the samples were calculated to be 3.9853 and 5.228 mg Catechol eq/ml, respectively.
- Sample 2 demonstrated significant results and clearer zone of inhibition against all the four bacteria.
- The percentage survival of the cell was recorded as 76.76% (Sample 1) and 78.26% (Sample 2), with IC50 values of 23.5111 ± 0.4474 & 23.0719 ± 0.4425 respectively.
- No hyperactivation of other cells in blood culture were recorded, indicating that these samples do not alleviate any hypercell activity and are safe for utilization on human blood cells.
- RBC membrane stabilization demonstrated 46.36% & 63.12% stabilization potentials for both the samples, indicating the anti-inflammatory potential of these samples.

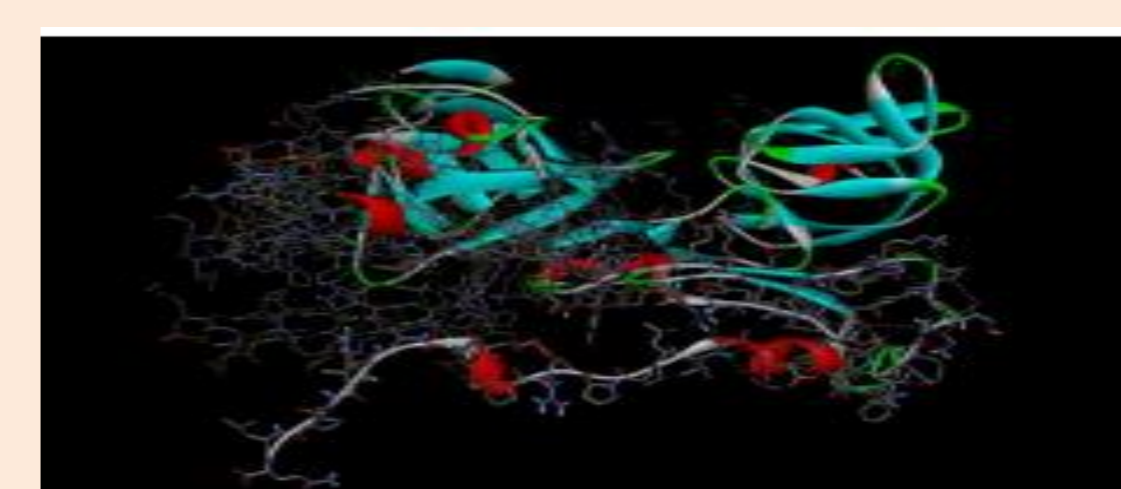
## In-silico analysis

### Ramachandran Plot data

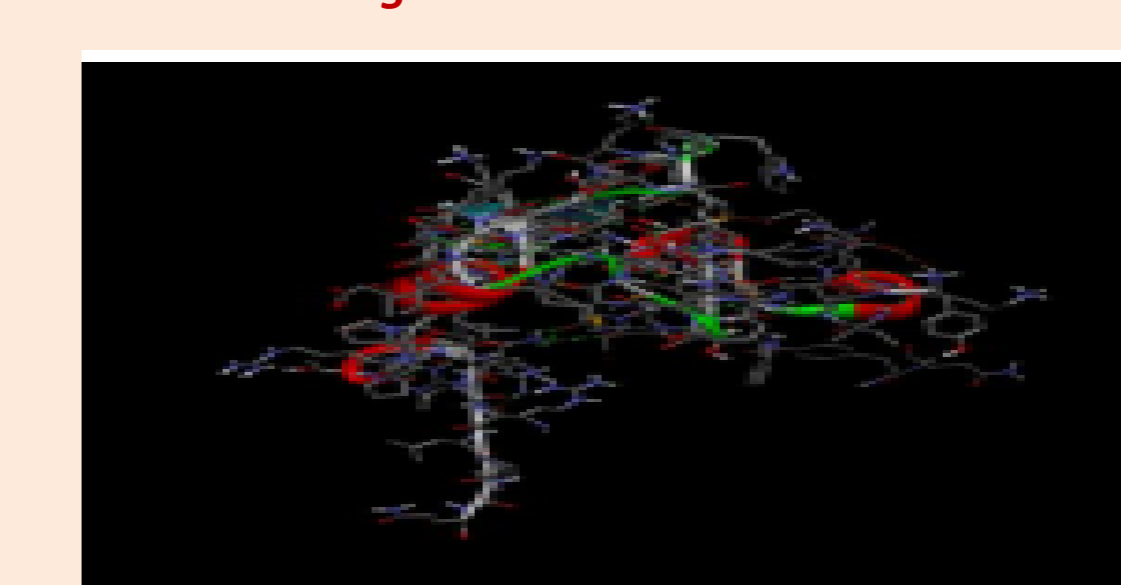
Protein	Highly preferred	Preferred
Cytochrome b (partial)	96.71%	2.82%
Cytochrome oxidase subunit 1, partial	97.02%	2.39%
long wavelength rhodopsin, partial	98.09%	0.67%
ringless gene (wgl)	91.54%	4.92%
elongation factor 1, alpha 2 gene	91.78%	4.11%
Arginine kinase gene	97.22%	2.78%
DNA topoisomerase 1 gene	99.25%	0.76%
Cytochrome C oxidase subunit 1, partial	98.18%	1.82%
Silk Fibroin	92.68%	2.44%



Protein-Protein complex of Cytochrome b oxidase from *P. falciparum* and the cytochrome b oxidase from *O. smaragdina*.

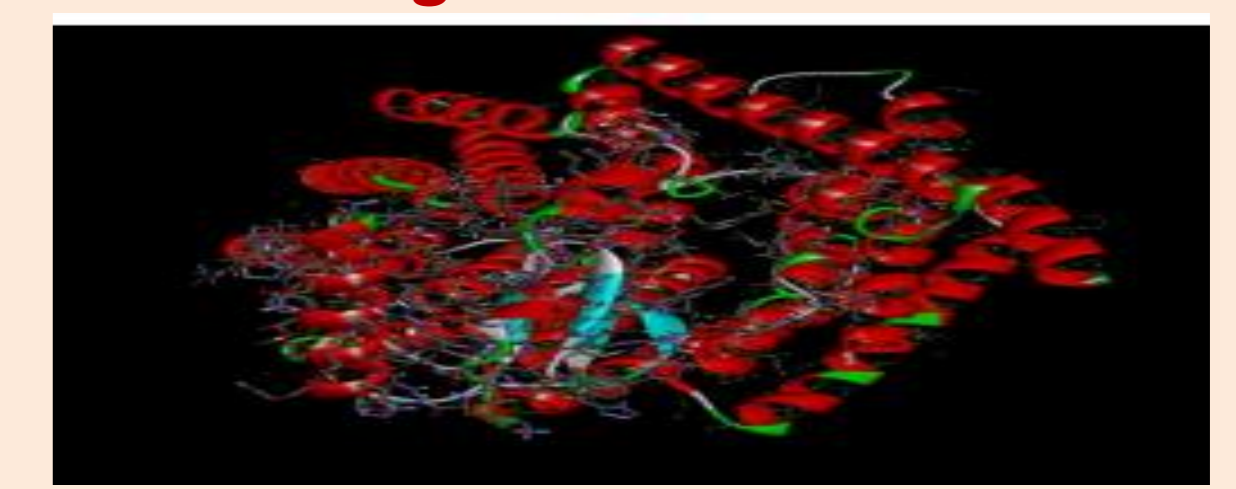


Protein-Protein complex of Elongation factor protein from *S. aureus* and the EF-1  $\alpha$ -2 from *O. smaragdina*.

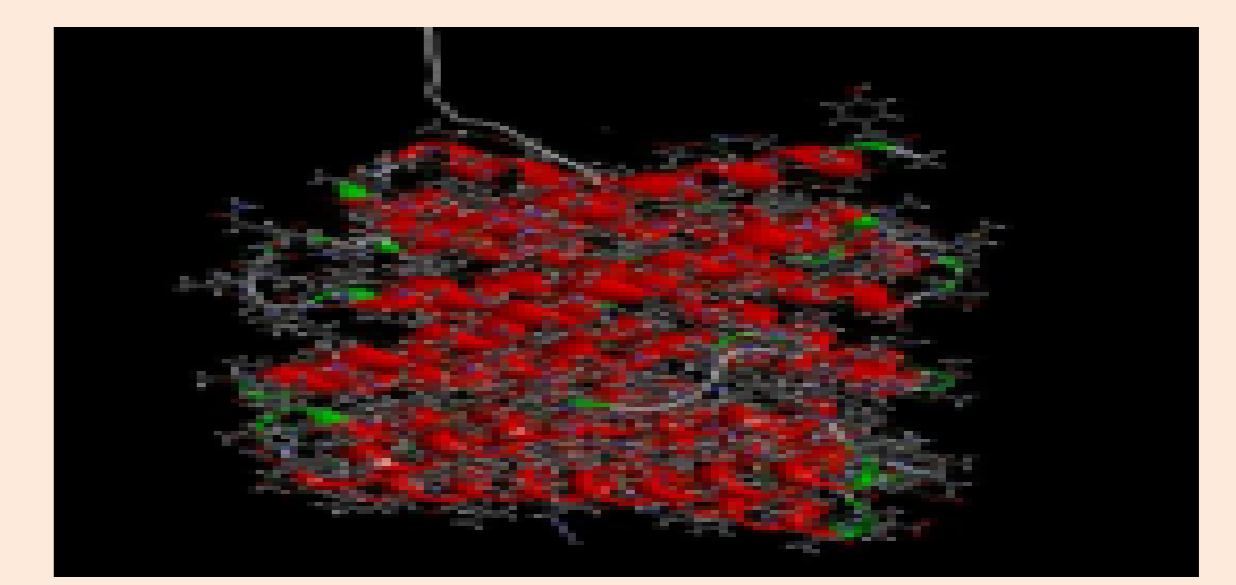


Protein-Protein complex of dengue virus corresponding genomic protein and the EF-1  $\alpha$ -2 from *O. smaragdina*.

## Protein-Protein Docking



Protein-Protein complex of Hepatitis B virus and the Silk fibroin protein from *O. smaragdina*.



Protein-Protein complex of SARS Cov-2'O ribose methyltransferase protein from SARS Cov-2 and the Silk fibroin from *O. smaragdina*.

## Conclusion

- Red weaver ants are great reservoir for proteins, calcium and zinc which were obtained in significant amounts in both the samples.
- Free polyphenols with higher radical scavenging activity was observed in the sample 2.
- Due to the presence of high formic acid, the samples exhibited increased anti-microbial potential.
- The cytotoxicity assay and the anti-inflammatory studies suggests the consumption of red weaver ants are SAFE for humans.
- Protein-protein docking studies reported a strong affinity between both the proteins. From the *in-silico* analysis, here we predict the plausible interaction between the proteins from the red weaver ants that interacts to the proteins known as drug target from the selected pathogen and hence may be able to inhibit their pathogenicity.

No Conflict of Interest: The authors have no conflict of interest

Acknowledgements: The authors thank the School of Biotechnology and Bioinformatics, D Y Patil Deemed to be University, India for all the facilities provided for research

## References:

- Wheeler DE, Martínez T. Storage proteins in ants. *Comp Biochem Physiol B Biochem Mol Biol*. 1995, 112(1):15-9. doi: 10.1016/0305-0491(95)00035-7.
- Shanmugam Alagappan, et al 2021. Nutritional analysis, volatile composition, antimicrobial and antioxidant properties of Australian green ants (*O. smaragdina*), *Future Foods*, 3,100007,https://doi.org/10.1016/j.fufo.2020.100007.