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

Background: The consumption of red weaver ant and their eggs 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. However, it still lacks a scientific evidence over the use.

Objective: The goal of the study was to perform and analyze the biochemical, antimicrobial, anti-inflammatory and cytotoxicity effects of the consumption of the red weaver ant and their eggs (*O. smaragdina*) and whether they are fit to consume by the humans imparting the potential to the treatment of claimed diseases.

Methodology: In the present study, two samples of the red weaver ants, namely from Bastar (Chhattisgarh) and Gadchirolli (Maharashtra) are compared. Both the sample were dried, grinded and soxhlet based extraction was carried out in the methanol solvent respectively. Crude proteins and elements (Zinc and Calcium) were determined at Bombay Test House, Vashi. 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 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 were optimized using GROMACS package and protein-protein docking was carried out with the drug target of the gastrointestinal (*E.coli*), respiratory (*S. aureus, S. pyogenes and K. pneumoniae*), Malarial (*Plasmodium*), Dengue, Jaundice and COVID-19 (SARS CoV-2) infectious pathogens using the computational tool, Hex 8.0.0.

Results:

Biochemical: 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.

Anti-microbial: The ant sample from Gadchirolli demonstrated significant results and clearer zone of inhibition against all the four bacteria, while the former sample gave clear zone of inhibition only for *E.coli* and bit for *K. pneumoniae.*

In-vitro: The cytotoxicity of the samples was evaluated *in-vitro* on PBMNCs culture. The percentage survival of the cell was recorded as 76.76% (Bastar) and 78.26% (Gadchirolli), with IC_{50} values of 23.5111± 0.4474 & 23.0719 ± 0.4425 respectively. No other hyperactivation of other cells in blood culture were recorded, indicating that these samples do not alleviate any hyper cell activity and are safe for utilization on human blood cells. Furthermore, analysis of the RBC membrane stabilization demonstrated the 46.36%) &

63.12% stabilization potentials of both the samples, indicating the anti-inflammatory potential of these samples.

In-silico: 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.