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Evaluation of Insecticidal Activity of Zoochemical-Assisted Zinc Oxide Nanoparticle Using Marine Invertebrate *Hyattella intestinalis* (Lamarck, 1814)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The term "zoochemicals" refers to animal chemical compounds, including secondary metabolites. Secondary metabolites are not only synthesised by plants and microorganisms, but animals are also naturally provided with valuable secondary metabolites, which are to be coined "Zoochemicals." In the present study, we aim to introduce the zoological term "zoochemical-assisted zinc oxide nanoparticle synthesis" using zoochemicals from the marine sponge *Hyattella intestinalis* and record marine invertebrates' zoochemicals as an important source of nanoparticle

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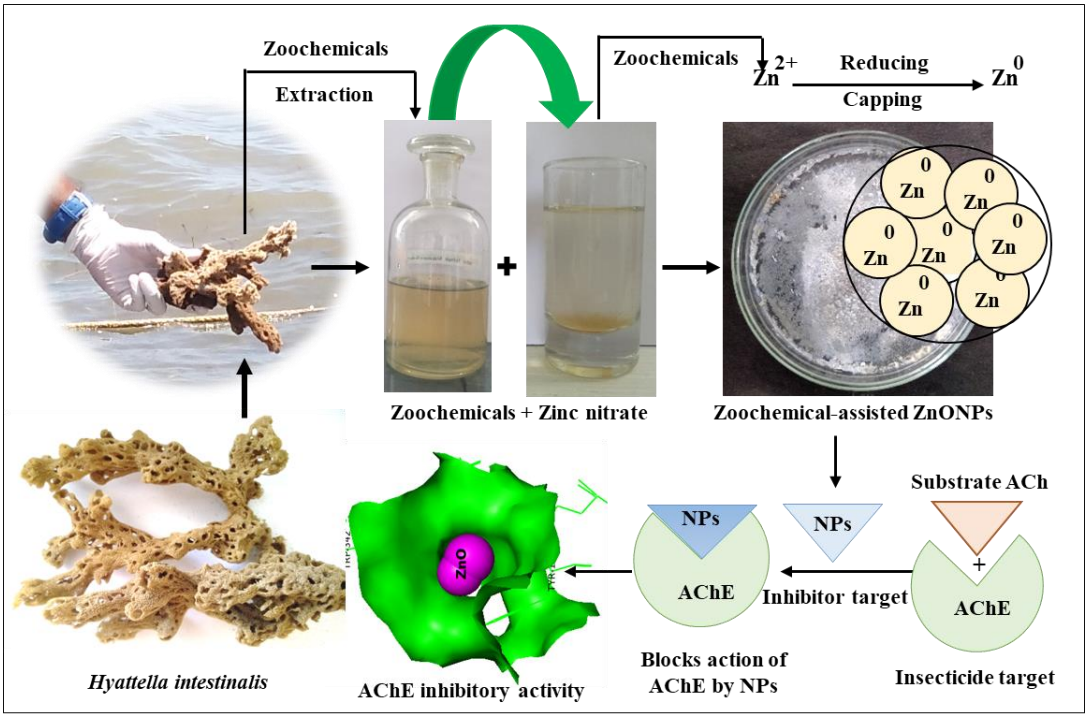
synthesis. In this terminology zoologically refers to zoochemicals from zoo-extracts that contain a varied range of animal secondary metabolites that involve the reduction of metal ions (M^+ to M^0) to form zoochemical-assisted metallic nanoparticles, which is similar to zoochemical-mediated nanoparticle synthesis. Finding the zoochemicals of animal origin to reduce zinc ions (Zn^{2+}) to the formation (Zn^0) of a brownish-yellow precipitate resulted in zoochemical-assisted ZnO nanoparticle synthesis. The UV-visible absorption peak at 378.90 nm initially confirmed Z-ZnONPs formation, and the FTIR spectrum revealed the presence of functional groups that are involved in reducing and capping agents of zoochemicals in NPs synthesis. The synthesized Z-ZnONPs had promising insecticidal activity through *in vitro* AChE inhibitory activity (IC_{50} = 129.07 µg/ml) with a correlation coefficient statistic agreed (R^2 = 0.9809), and computational investigation was supported by ZnO interaction with the target insecticide AChE (energy values = -59.42 Kcal/mol, using Hex). The present study is a scientifically first-hand report of "zoochemical-assisted ZnONPs nanoparticle synthesis" using zoochemicals from the marine invertebrate *Hyattella intestinalis* as reducing and capping agents in nanoparticle synthesis and eco-friendly nanodrugs.



Graphical abstract of zoochemical-assisted zinc oxide nanoparticles synthesis using marine invertebrates *Hyattella intestinalis* and its evaluation of insecticidal activity

Keywords: Zoochemicals; zoochemical-assisted; zinc oxide nanoparticle; Hyattella intestinalis; insecticidal activity.

ABBREVIATIONS

Zn^{2+} : Zinc ions
Z-NPs : Zoochemical-assisted nanoparticles
ZnO : Zinc oxide
Z-ZnONPs : Zoochemical-assisted zinc oxide nanoparticles
AChE : Acetylcholinesterase
ACh : Acetylcholine
N : Number of replicates

1. INTRODUCTION

The term "zoochemicals" refers to animal chemical compounds, including secondary metabolites. Secondary metabolites are not only synthesized by plants and microorganisms, but animals are also naturally provided with valuable secondary metabolites, which are to be coined "zoochemicals." Animal secondary metabolites, such as alkaloids, terpenoids, sponins, etc., are

similar to plant secondary metabolites [1]. The zoochemicals, including animal secondary metabolites, are involved in reducing metal ions to form metallic nanoparticles, in this terminology to be coined zoochemical-mediated nanoparticle synthesis [2,3], which zoochemicals are responses to reducing and capping agents. Nanoparticle synthesis methodology used chemical and physical techniques, but these techniques were too expensive and not beneficial to the environment [4]. Dos Santos et al. [5] recommend the green nanoparticle synthesis technique. The green synthesis of nanoparticles refers to three main conditions: environmental solvents, good reducing agents, and harmless material for stabilization [6]. The present study followed the green synthesis of metallic nanoparticles, and I used an aqueous solvent, natural reducing agents (secondary metabolic), and zoochemicals extracted from animal sources of marine sponge.

Storage pests have a direct impact on the quantity and quality of grain during postharvest storage, resulting in huge economic losses. The increase in yield of agricultural products associated with the green revolution [7] is possible in part because of the discovery and utilization of synthetic chemicals for insecticides. Phytophagous insects are a major source of agricultural loss [8] and require the use of synthetic insecticides, which result in the control of harmful insects but are highly toxic for humans. Dayan et al. [7] report that a succession of new pesticides based on natural products are being developed to replace the synthetic compounds, reducing the number of synthetic pesticides available in agriculture. Today, globally, new insecticides are discovered that, armed with new tools, provide the future of insect control [9]. For instance, metallic nanoparticles have recently attracted research attention as pesticides, which are synthesized from natural resources by biological methods [8]. The aim of the present study is zoochemical-assisted zinc oxide nanoparticle synthesis and characterization using marine invertebrates, *Hyattella intestinalis* (Lamarck, 1814), and its evaluation of insecticidal activity.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Zoochemicals from *Hyattella intestinalis*

The marine sponge *Hyattella intestinalis* (Lamarck, 1814) was collected from the east coast of Mallipattinam village, Thanjavur district, Tamil Nadu, India, during - 2021. The collected marine sponge was identified by a literature of Sivaleela, [10], and the molecular taxonomic identification (Accession No. OQ196103). *Hyattella intestinalis* zoochemicals were extracted with deionized water using the Soxhlet apparatus, as reported by Karnan et al. [1]. The 30 grams of *Hyattella intestinalis* marine sponge were extracted using the Soxhlet apparatus with 300 ml of deionized water and followed by a time duration of 3 h with temperature contact of 50 to 60 °C to obtain a brownish-colored extract that was reduced to 30 ml. The concentrated zooextract that zoochemicals contain is used in zoochemical-assisted nanoparticle synthesis.

2.2 ZnO Nanoparticles Synthesis

The present study I have introduced the term “zoochemical-assisted nanoparticle synthesis” as similarly phytochemical-assisted nanoparticle synthesis, and the present study's methodology is supported by the method of “green synthesis” of zinc oxide nanoparticles by Selim et al. [11] with minor modification.

2.3 Zoochemical-assisted Nanoparticle Synthesis

The crude zoo-extract from 30 grams of *Hyattella intestinalis* marine sponge was extracted using the Soxhlet apparatus with 300 ml of deionized water and followed by a time duration of 3 h with temperature contact of 50 to 60 °C to obtain a brownish-colored extract that was reduced to 30 ml. The 25 ml of zoo-extract was heated and the extract reached 60 °C using a magnetic stirrer with zoochemicals contained and added to 2.5 g of zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$), which contained 0.53 M $Zn(NO_3)_2.6H_2O$, and continued stirring for 30 minutes using the magnetic stirrer. It took 1 hour to form a brownish-yellow precipitate in the bottom of the glass beaker. This precipitate was collected and washed several times with a solution of distilled water. Afterwards, the collected precipitate was transferred to a ceramic crucible cup and heated in furnace at 400°C for 1 h [11]. The resultant white powder (Z-ZnONPs) stored in an airtight container for characterized using spectroscopy techniques.

2.4 *In vitro* AChE Inhibition Assay

Previously, Karnan et al. [2] reported *in vitro* AChE inhibitory activity of *Hyattella intestinalis* zoo-extract, followed by a method of *in vitro* AChE inhibition assay by Ellman et al., [12] with

a mildly modified and present study *in vitro* evaluation of AChE inhibitory activity using zoochemical-assisted ZnONPs from *Hyattella intestinalis* zoochemicals. Data (N = 3) were analyzed by IBM SPSS Version 20.0. The IC_{50} values (with 95% confidence limits) were calculated. Also, find the linear regression equation and the correlation coefficient (r) statistic.

2.5 Molecular Docking

Computational drug discovery technique successfully molecular modeling with different algorithm based programing software's been used. The ligand and protein binding scores according to algorithm based program thereby may use any software for protein and ligand interactions for best results [13]. ZnO was obtained from the PubChem database, and storage pest *Tribolium castaneum* acetylcholinesterase (AChE) homology modeling was used using the Swiss model (GenBank: EEZ99262.2). The ZnO was docked with the target AChE receptor using Hex programs with default parameters and docked complexes were visualized with the PyMOL 2.3.1 tool.

3. RESULTS AND DISCUSSION

3.1 Zoochemical-Assisted ZnONPs Synthesis

The present study, carried out to introduce zoological term "zoochemical-assisted ZnONPs synthesis" using zoochemicals from *Hyattella intestinalis* as reducing and capping agents, including animal secondary metabolites, such as alkaloids, terpenoids, sponins, etc., are similar to plant secondary metabolites [1]. Previous *Hyattella intestinalis* zoochemicals contained alkaloids and terpenoids, and spectroscopic techniques revealed 60 zoochemicals using GC-MS [1]. The HPLC techniques revealed alkaloids derivatives, and the FTIR spectrum agreed on amine functional groups [3]. The presence of *Hyattella intestinalis* zoochemicals is involved in metal ion-reducing agents that form metallic nanoparticles. Finding the result zoochemicals from *Hyattella intestinalis* as reduced Zn^{2+} ions to the formation (Zn^0) of a brownish-yellow precipitate resulted in a zoochemical-assisted ZnO nanoparticle (Fig. 1). This collected precipitate was heated in a furnace at 400°C for 2 hours. The resultant white powder (zoochemical-assisted ZnONPs) stored in an airtight container was characterized using spectroscopy techniques.

Similarly, the present study, supported by Selim et al. [11], has studied ZnONP synthesis using an aqueous extract of *Deverra tortuosa;* that extract was reduced zinc nitrate, which resulted in a change in the color of the solution and the formation of a yellowish-white precipitate. Selim et al. used 25 ml of *Deverra tortuosa* extract heated (60–80°C) on a magnetic stirrer to reach a temperature of 60°C, and 2.50 g of zinc nitrate hexahydrate was added after 1 hour until a white precipitate appeared. Afterwards, the collected paste was heated in a furnace at 400°C for 2 hours, resulting in white powder ZnONPs [11]. Iqbal et al. [14] reported the green synthesis of zinc oxide nanoparticles using *Elaeagnus angustifolia* extracts. Iqbal et al. used 1 g of zinc-nitrate hexahydrate and added 100 mL of *E. angustifolia* leaf extract. The mixture was continuously heated at 60°C for 2 hours. After the solution color turned yellowish black, indicating ZnONPs, the synthesized solution was centrifuged at 5000 rpm for 20 min, and the obtained residue (ZnONPs) was dried in an oven at 100°C for 3 h in Petri dishes [14]. About the literature supported by the present result of zoochemical-assisted ZnONPs synthesis using *Hyattella intestinalis*.

Animal secondary metabolites are naturally reduced metal ions and reducing agents, including marine invertebrate animals such as the marine sponge *Hyattella intestinalis.* Previously, Karnan et al. [2,3] recorded zoochemicals from the marine sponge *Hyattella intestinalis* that reduced copper ions (Cu^{2+}) to the formation (Cu^0) of zoochemical-mediated copper oxide nanoparticles. In the current study, another terminology was introduced for the synthesis of zoochemical-assisted ZnONPs using zoochemicals from marine sponge *Hyattella intestinalis* as reduced Zn^{2+} ions to the formation (Zn^0) of zoochemical-assisted ZnO nanoparticles as a similarly termed zoochemical-mediated mechanism. Zoochemical-assisted ZnO nanoparticle formation was initially agreed upon on the UV-visible spectrum, which revealed the peak at 378.90 nm (Fig. 2). The FTIR spectrum of zoochemical-assisted ZnO nanoparticles revealed the presence of functional groups, such as alcohols, phenols, carboxylic acids, alkanes, amines and alkynes, to confirm the presence of secondary metabolic zoo-compounds, which are involved in reducing and capping agents of zoochemicals to form Z-ZnONPs, as represented in Table 1 and Fig. 3.

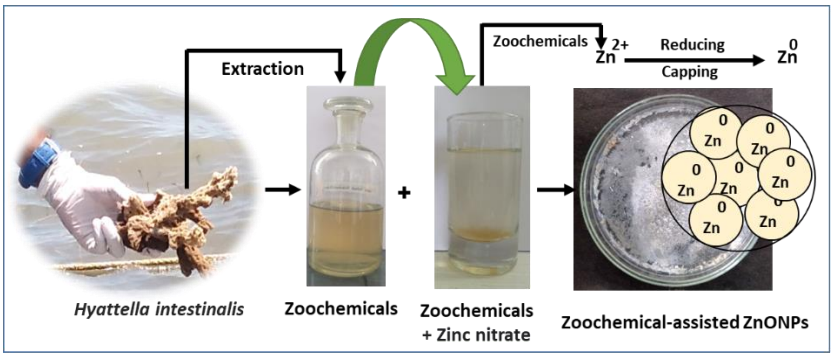


Fig. 1. The term "zoochemical-assisted ZnONPs synthesis" using zoochemicals from *Hyattella intestinalis*

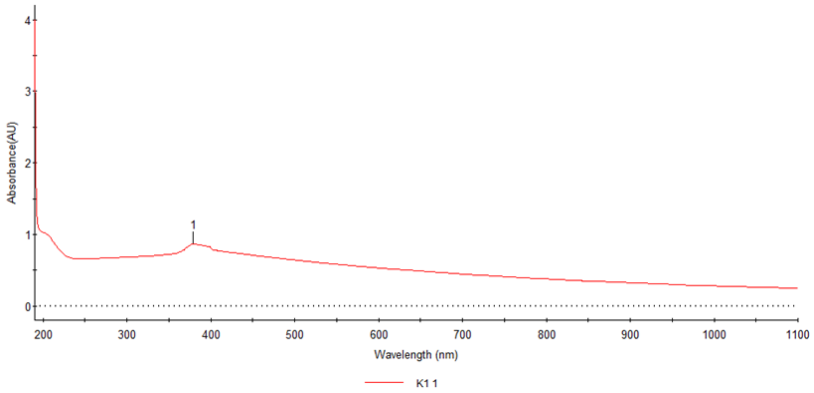


Fig. 2. UV-Visible analysis of zoochemical-assisted ZnO nanoparticle (Peak at 378.90 nm)

Table 1. The FTIR analysis of zoochemical-assisted synthesised ZnO nanoparticles

Frequency cm^{-1}	Bond	Functional group
3437.98	O-H stretch, H-bonded	Alcohols, Phenols
2921.04	O-H stretch	Carboxylic acids
2851.80	C-H stretch	Alkanes
1633.02	N-H bend	1° amines
1248.60, 1107.11	C-N stretch	Aliphatic amines
618.17	-C≡C-H: C-H bend	Alkynes

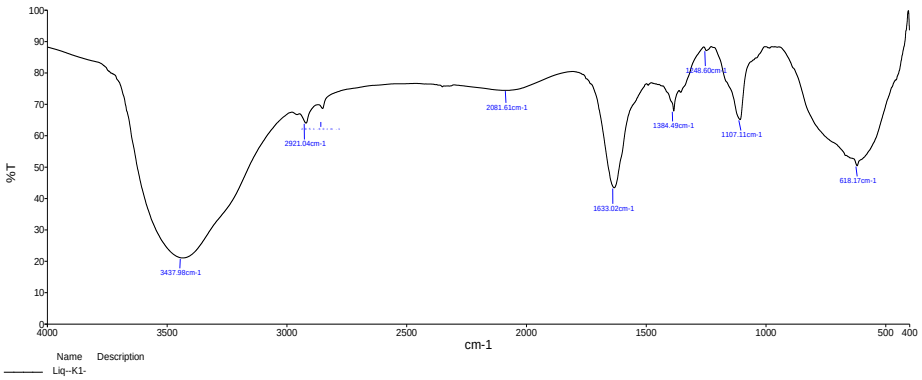


Fig. 3. FTIR spectrum of zoochemical-assisted synthesised ZnO nanoparticles

Selim et al. [11] reported that ZnONPs were initially confirmed by a UV-visible technique that revealed a characteristic peak at 374 nm using the plant reducing agent *Deverra tortuosa,* and similarly, Raja et al. [15] observed an absorption peak at 376 nm from ZnONPs using the reducing agent *Hyptis suaveolens* leaf extract. Ahmad and Kalra, [16] similarly supported UV-Vis spectroscopy, which revealed an absorption peak at 370 nm of ZnO nanoparticles, and the FTIR spectrum agreed on the presence of functional groups responsible for the reduction in NP synthesis. Even Muthu Kathija et al. [17] studied FTIR analysis of ZnO NPs with the presence of functional groups that were involved in reducing and stabilizing agents during the formation of ZnO NPs. The UV-visible technique was initially used to confirm the formation of ZnONPs [18]. Ashwini et al. [19] used zinc nitrate hexahydrate, which is added to *Acacia caesia* bark aqueous extract and heated to form a yellow color that serves as a visual confirmation, and preliminary confirmation of zinc oxide was carried out by UV-visible spectroscopy [19]. FTIR analysis determines the functional groups contained in the reducing agents as well as their role in the stabilization and synthesis of ZnONPs [20]. FT-IR and UV-vis spectroscopy spectra indicate the presence of ZnONPs [21]. The present work is a scientific first-hand report of the term "zoochemical-assisted ZnONPs synthesized" using zoochemicals from the marine sponge *Hyattella intestinalis,* and the zoochemical-assisted terminology is similar to the phytochemical-assisted term.

3.2 *In vitro* and *In silico* Insecticidal Activity of Zoochemical-Assisted ZnONPs, through AChE Inhibitory Activity

Acetylcholinesterase (AChE) is a target of insecticide and is a cholinergic enzyme. It immediately breaks down or hydrolyzes acetylcholine into acetic acid and choline, a naturally occurring neurotransmitter [22]. They are important inhibitors of AChE in the development of insecticides and their toxicity by inhibiting the AChE enzyme, leading to their death in insects [23]. The synthesized Z-ZnONPs had promising insecticidal activity through *in vitro* AChE inhibitory activity (IC_{50} = 129.07 µg/ml) with a correlation coefficient statistic agreed (R^2 = 0.9809), represent in Fig. 4 and *Hyattella intestinalis* zoo-extract *in vitro* AChE inhibitory activity was (AChE IC_{50} = 141.10 µg/ml) previous report by Karnan et al. [2]. Shunmuga Vadivu and Velavan, [24] reported that the enzyme inhibitory effect may be attributed to the presence of secondary metabolites in plants and animal secondary metabolites equal to plant secondary metabolites [1]. Similar results were observed in our study due to animal secondary metabolites involved in enzyme inhibition. The computational study supported the AChE inhibitory activity of Z-ZnONPs using molecular docking. The present molecular docking study agreed that Z-ZnONPs inhibit the AChE enzyme (energy values = -59.42 Kcal/mol, using Hex software), as represented in Fig. 5. The AChE binding site of target amino acids in Glu 112, Arg 113, Tyr 114, and Trp 342 by binding interaction with ZnO (Table 2). Its zoochemical-assisted ZnONPs inhibited acetylcholinesterase to increase acetylcholine in synoptic cells, causing overstimulation of ACh receptors and symptoms of poisoning against insect pests.

Ishwarya et al. [25] studied the insecticidal activity of ZnONPs using *Ulva lactuca* seaweed against *Aedes aegypti* fourth instar larvae mortality (100%) was observed within 24 h at a concentration of 50 µg/ml, and acetylcholine esterase was significantly decreased after larvae fed on LC_{50} of green synthesized AgNPs and ZnONPs from *Moringa oleifera* leaf extract, treated diets as compared with the control [26], which confirms the insecticidal active properties of ZnONPs. Even El Gohary et al. [27] reported that nano-encapsulated EO decreased acetylcholinesterase when controlling *Culex pipiens* larvae. Gutiérrez-Ramrez et al. [28] coined nanoparticles as an alternative pest control and studied the insecticidal effect of zinc oxide nanoparticles (ZnO NPs) and titanium dioxide nanoparticles (TiO_2 NPs) against *Bactericera cockerelli* (Hemiptera: Triozidae) second-stage nymphs. The computational study agreed by Benslama et al. [29] to find out the inhibitory potential of its polyphenolic compounds against the acetylcholinesterase enzyme template of chain A in 5YDJ of the acetylcholinesterase protein from *Anopheles gambiae* insects. Above the ZnONPs insecticidal properties was supported evidence of the present zoochemical-assisted ZnONPs *in vitro* AChE inhibitory activity, which results in Z-ZnONPs inhibiting acetylcholinesterase to increase acetylcholine in synoptic cells, causing overstimulation of ACh receptors and symptoms of poisoning against insect pests.

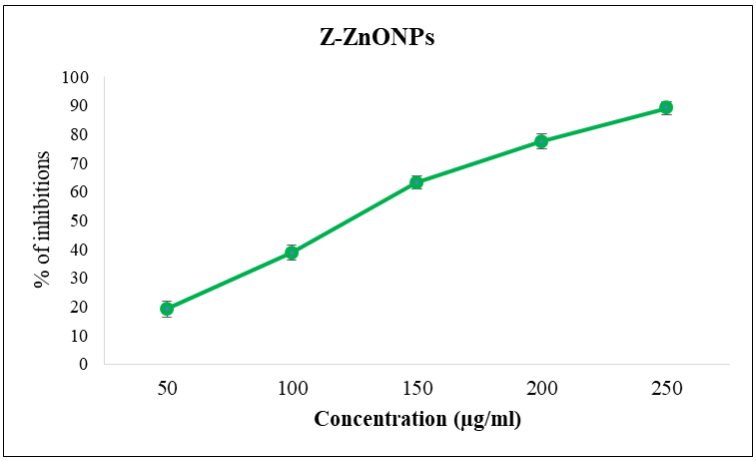


Fig. 4. *In vitro* insecticidal activity of zoochemical-assisted ZnONPs, through AChE inhibitory activity

Table 2. Molecular docking assessment of ZnO and storage pest *Tribolium castaneum* acetylcholinesterase (AChE) homology modeling (GenBank: EEZ99262.2)

ZnO	Energy values (Kcal/mol)	ZnO interaction with target protein amino acid residues
AChE	-59.42	Glu 112, Arg 113, Tyr 114, Trp 342.

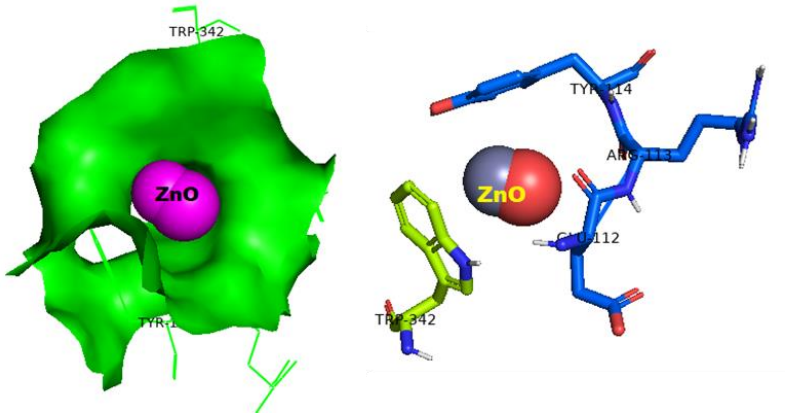


Fig. 5. ZnO binding interaction with AChE using molecular docking

4. CONCLUSION

The synthesized Z-ZnONPs had promising insecticidal activity through *in vitro* AChE inhibitory activity and computational investigation was supported by ZnO interaction with the target insecticide AChE. The marine invertebrate zoochemicals from *Hyattella intestinalis* are scientific evidence of zinc ion-reducing and capping agents in zinc oxide nanoparticle synthesis. The present work is a scientific first-hand report of the term “zoochemical-assisted ZnONPs synthesized” using zoochemicals from the marine sponge *Hyattella intestinalis*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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