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SHORT COMMUNICATION

# Influence of heat shock protein (HSP-70) enhancing compound from red alga (*Porphyridium cruentum*) for augmenting egg production in copepod culture - A new *in silico* report

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ABSTRACT

The present study reports *in silico* investigation of bioactive compounds from marine microalgae capable of escalating copepod fecundity potential through enhanced heat shock protein (HSP-70) production. The structure of ligand (bioactive compounds from microalgae) and hsp-70 obtained from the databases of PubChem and Protein Data Bank (PDB), respectively. Molecular Docking was performed by GOLD software and ligand interaction pathways using web server MANORAA. Fourteen bioactive compounds showed good binding interaction with specific protein HSP-70 and seven of these compounds showed high hydrogen bond interaction with key amino acids (phenylalanine, tyrosine and tryptophan). The highest binding energy of 50.21is recorded in the bioactive compound, arachidonic acid from the red alga *Porphyridium cruentum* TYR 167 involved in the biosynthesis pathway of phenylalanine, tyrosine and tryptophan also showed specific target site of tryptophan synthase (4.2.1.20). Results suggest with *P. cruentum* feed copepod culture could boost their fecundity leading to high density culture.

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## Introduction

Marine food webs depend on zooplankton especially, copepods to process and repackage energy harnessed by photosynthetic primary producers. Copepods constitute important primary consumer in all types of aquatic ecosystems and play vital role in the energy transfer from primary producers to secondary consumers (Altaff, 2020). In aquaculture, copepods have been proven to be the preferred and most adequate food for fish larvae (Anandan et al., 2013;

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Vu et al., 2017; Hansen, 2017). The nutritional quality of copepods is accepted to be highly satisfactory for larvae of prawn and finfish species. Biochemical studies have shown that copepods are rich in proteins, lipids, essential amino acids, and essential fatty acids which can enhance reproduction, augment growth, immune stimulation, and color intensification in prawn and fish larvae (Lavens & Sorgeloos, 1996; Aman & Altaff, 2004). For this reason, copepods are cultivated for use as live feed for newly hatched finfish larvae in marine aquaculture systems. Copepods in cultivation systems may be exposed to environmental conditions such as fluctuations in temperature, pH and pathogens that challenge successful large scale production (Petkeviciute et al., 2015). Therefore, it is essential that copepod species chosen for mass culture should be resistance to such environmental conditions. The egg productions of marine copepod may be under favorable or unfavorable environmental conditions. Under favorable conditions, subitaneous eggs are produced that are characterized by hatching within a few days after spawning (Nilsson et al., 2014). In response to adverse environmental conditions, subitaneous eggs enter a quiescent state (direct inhibition of development due to adverse conditions), where embryonic development is delayed until exposure to more favorable environmental conditions. When a copepod embryo undergoes quiescence, it requires a number of stabilizing factors (Nilsson et al., 2014).

The heat shock protein 70 (HSP-70) production is the response of copepods in shallow waters protects them against the adverse environmental conditions such as temperature and pH which otherwise leads to damage the cellular macromolecules through ROS - reactive oxygen species (Nilsson et al., 2014). Aruda et al. (2011) also reported that the HSP-70 of copepod in shallow waters protects proteins against the higher temperatures experienced under these environmental conditions. However, only few copepod species have so far been subject of such *in vivo* studies among the marine copepods. *Acartia tonsa* response to the heat shock was more pronounced at low salinity model (Nilsson et al., 2014; Petkeviciute et al., 2015) and similar impact of sublethal stress of *A. tonsa* using solar UV radiation was also reported (Tartarotti & Torres, 2009). Likewise, Rhee et al. (2009) reported that the HSP-*70* gene expression is elevated when copepods are exposed to elevated temperatures. Based on the above rational, in the present study an attempt is made to search for HSP-70 enhancement bioactive compounds from microalgae using *in silico* modeling. These molecular interaction studies are not reported to our knowledge in any copepod. Present study aims to enhance and regulate fundamental cellular processes during high density culture of marine copepod especially, through biosynthesis of amino acid (Phenylalanine, tyrosine and tryptophan biosynthesis).

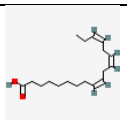
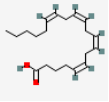
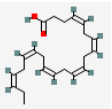
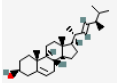
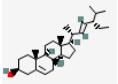
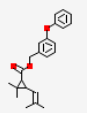
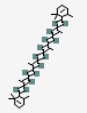
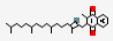
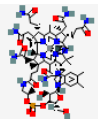
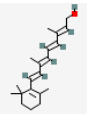
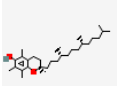
## Material and Methods

The molecular docking studies were performed using GOLD software. The structures of bioactive compounds were built using ChemDraw program (Table-1) then were transferred into Discovery Studio 2.5 (Accelrys Inc, San Diego, CA, USA). Compounds were typed with CHARMM force field and partial charges were calculated by Momany-Rone option. The resulting structures were minimized with Smart Minimizer algorithm which performs 1000 steps of steepest descent with a RMS gradient tolerance of 3, followed by Conjugate Gradient minimization. In order to investigate the binding mode of inhibitors, and obtaining nearly bioactive conformations, X-ray crystal structure of HSP-70 complex was taken from PDB (3P9Y) with its resolution 2.10 Å and used for docking studies. The ability of GOLD to produce original ligand binding orientations is greater than 70%. The active site in the HSP-70 protein complex, crystal coordinate was defined as a region with a radius of 10 Å. Preparation for docking process; water molecules were removed; hydrogen atoms were added in the receptor using GOLD. Reproducibility of docking program was checked and compared with original crystal structure. It yields the RMS value of 0.95 Å. This result conform the reproducibility of GOLD program. The early termination option was used to skip the genetic optimization calculation when any five conformations of a particular compound predicted above the rmsd value of 1.5 Å. The best lead molecules were selected based on their binding orientation in the active site and their corresponding GOLD score. Based on molecular docking analysis the highest binding energy compound was selected for further ligand interaction pathway study and investigated with specific biosynthesis of amino acid pathway of phenylalanine, tyrosine and tryptophan using web server MANORAA - Mapping Analogous Nuclei onto Residue and Affinity (http://www.manoraa.org/).

## Results and Discussion

Molecular docking is an effective and competent tool for *in silico* screening. Docking is a computational procedure of searching for an appropriate ligand that fits both energetically and geometrically with the protein's binding site (Vijayaraj et al., 2019). During the past decade, for understanding the formation of intermolecular complexes, the application of computational methods has been subjected to intensive research. It is commonly known that molecular binding of

**Table 1.** List of ligand (Bioactive compounds from microalgae)

| Name of the ligands | Molecular formula | Molecular structure | Ligand sources | Reference |
|---|---|---|---|---|
| α-Linolenic acid | $C_{18}H_{30}O_2$ |  | *Arthrospira* sp. | Cohen & Heimer, 1992 |
| Arachidonic acid | $C_{20}H_{32}O_2$ |  | *P. cruentum* | Ahern et al., 1983 |
| Docosahexaenoic acid | $C_{22}H_{32}O_2$ |  | *I. galbana* | Pulz & Gross, 2004 |
| Brassicasterol | $C_{28}H_{46}O$ |  | *Chaetoceros* | Bandarra et al., 2003 |
| Stigmasterol | $C_{29}H_{48}O$ |  | *I. galbana* | Tsitsa et al., 1993 |
| Pyocyanine | $C_{13}H_{10}N_2O$ |  | *Arthrospira* sp. | Mao et al., 2005 |
| Phenothrin | $C_{23}H_{26}O_3$ |  | *P. cruentum* | Pulz & Gross, 2004 |
| β-Carotene | $C_{40}H_{56}$ |  | *D. salina* | Plaza et al., 2009 |
| Ascorbic acid | $C_6H_8O_6$ |  | *Arthrospira* sp. | Antia et al., 1970 |
| Phylloquinone | $C_{31}H_{46}O_2$ |  | *P. cruentum* | Bandarra et al., 2003 |
| Cyanocobalamin | $C_{63}H_{89}CoN_{14}O_{14}P$ |  | *Pavlova* sp. | De Roeck-Holtzhauer et al., 1991 |
| Retinol | $C_{20}H_{30}O$ |  | *I. galbana* | Li & Xu, 2008 |
| α-Tocopherol | $C_{29}H_{50}O_2$ |  | *Pavlova* sp. | Morse et al., 1979 |
| γ-Aminobutyric acid | $C_4H_9NO_2$ |  | *Porphyridium* sp. | Obrietan et al., 2002 |

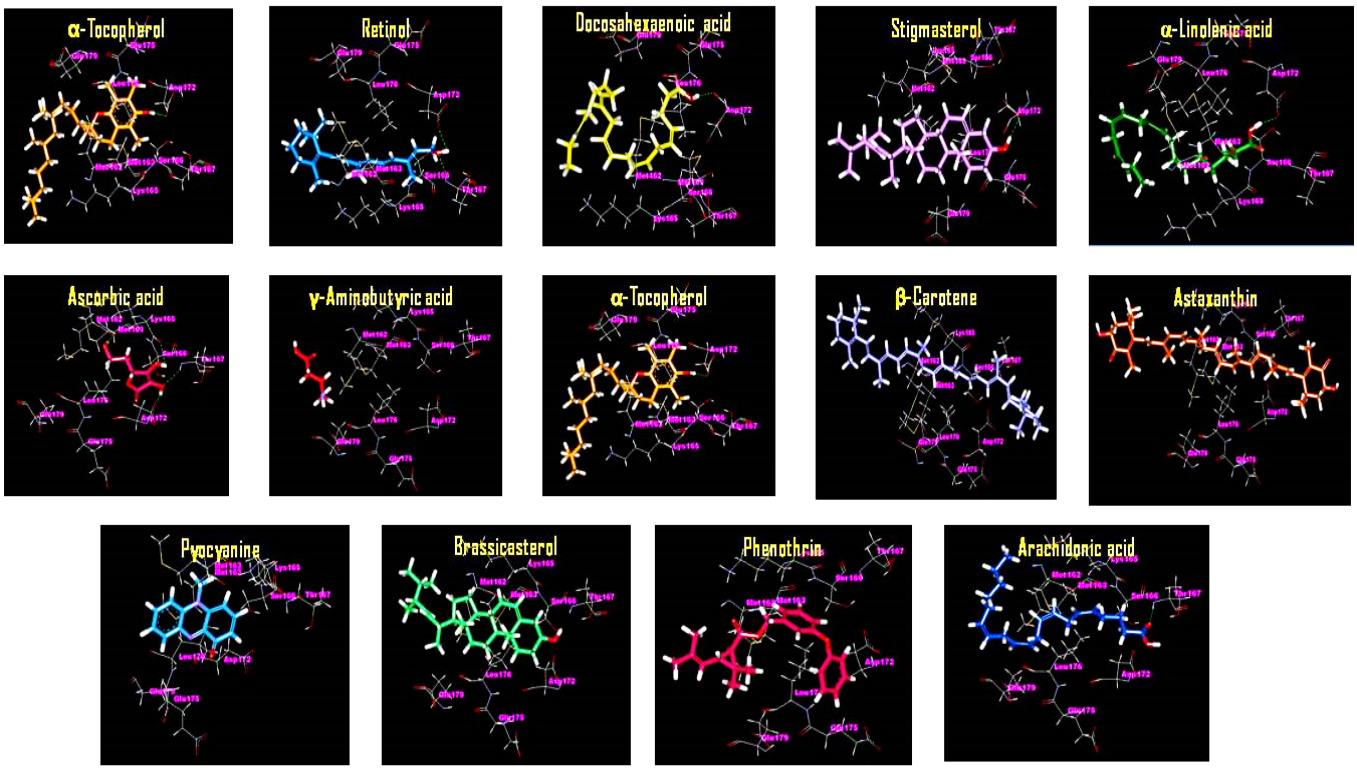



**Figure 1.** Bioactive compounds binding interaction with specific protein *hsp*-70

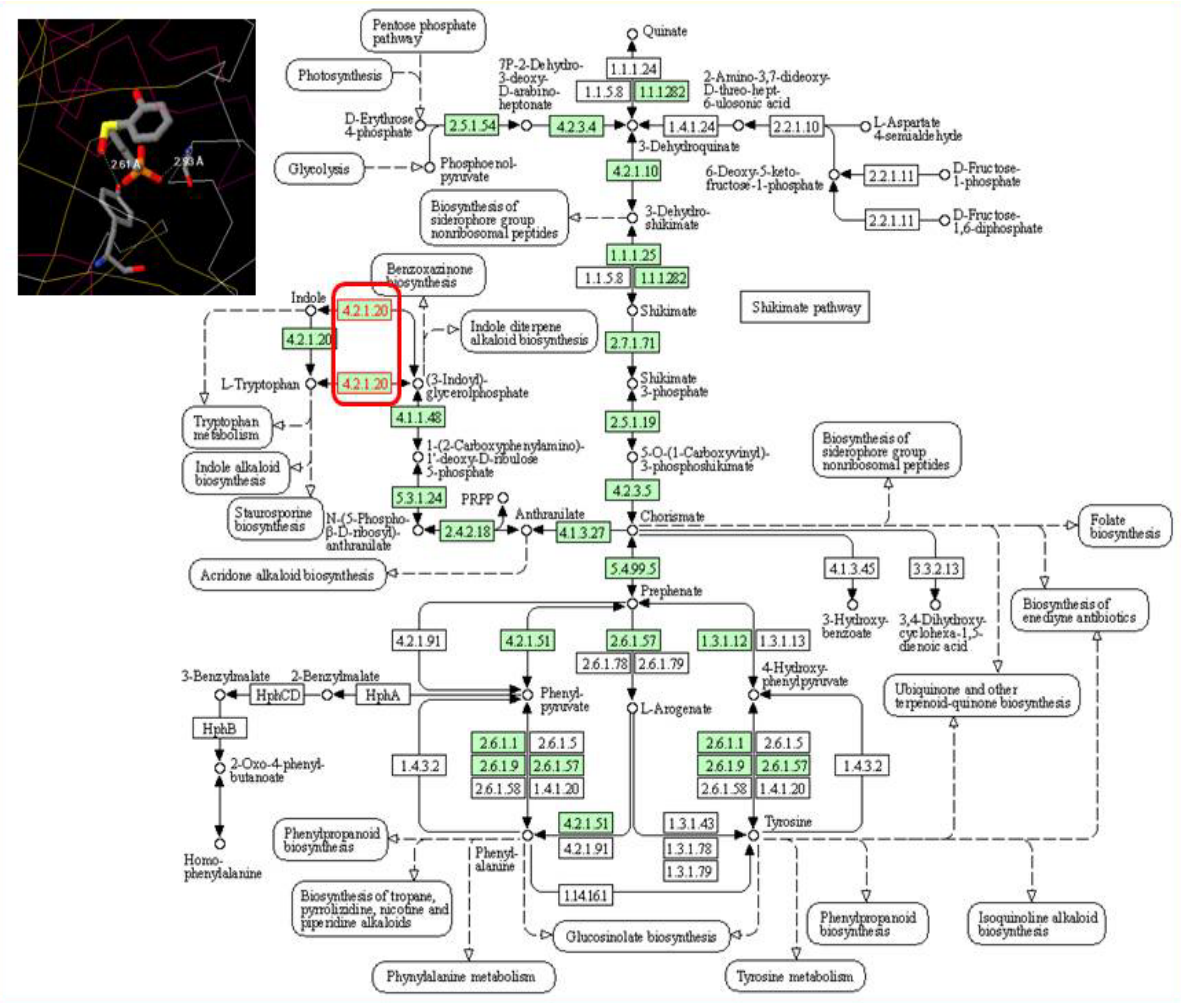



**Figure 2.** Arachidonic acid influenced phenylalanine, tyrosine and tryptophan biosynthesis pathway

bioactive compounds (ligand) to the pocket of another molecule (protein as a receptor) is responsible for accurate activity of the bioactive compounds. Molecular docking has been proved very efficient tool for novel discovery for targeting protein (Vijayaraj et al., 2020). In the present study, fourteen different HSP-70 enhancements by natural bioactive compounds from various microalgae were investigated. These compounds include α-Linolenic acid from *Arthrospira* sp., Arachidonic acid from *P. cruentum*, Docosahexaenoic acid from *I. galbana*, Brassicasterol from *Chaetoceros* sp., Stigmasterol from *I. galbana*, Pyocyanine from *Arthrospira* sp., Phenothrin from *P. cruentum*, β-Carotene from *D. salina*, Ascorbic acid from *Arthrospira* sp., Phylloquinone from *P. cruentum*, Cyanocobalamin from *Pavlova* sp., Retinol from *I. galbana*, α-Tocopherol from *Pavlova* sp. and γ-Aminobutyric acid from *Porphyridium* sp. (Raposo et al., 2013).

All the fourteen bioactive compounds showed good binding interaction with specific protein *hsp-70* (Figure-1). Among these seven bioactive compounds showed good hydrogen bond interactions with key amino acid (Table 2). The binding energy of the bioactive compound is α-Tocopherol (34.67), Arachidonic acid (50.21), Retinol (34.21), Docosahexaenoic acid (49.18), Phenothrin (41.36), Phylloquinone (42.05), β-Carotene (32.37), Stigmasterol (31.69), α-Linolenic acid (43.81), Astaxanthin (24.83), Brassicasterol (27.90), Ascorbic acid (24.32) and Pyocyanine (32.14). The bioactive compound, Arachidonic acid from microalgae, *Porphyridium cruentum* exhibited highest potential for enhancement of HSP-70 protein compared to other bioactive compounds.

To our knowledge there is no published report in copepods on target enhancement of cellular pathway especially for phenylalanine, tyrosine and tryptophan biosynthesis pathway. Because these three aromatic amino acids are involved in protein synthesis and synthesis of a variety of secondary metabolites a subset of which are involved in numerous anabolic pathways responsible for the synthesis of pigment compounds, hormones and biological polymers which are against reactive oxygen species in the organisms (Parthasarathy et al., 2018). In the present study, the bioactive compound, arachidonic acid is showing highest binding energy 50.21 kcl/mol with specific hydrogen bond interaction with amino acid is TYR 167. The tyrosine - 167 is potential signaling therapeutically importance against ROS (Budiman et al., 2004). The bioactive compound, arachidonic acid shows the potential enhancement of phenylalanine, tyrosine and tryptophan biosynthesis pathway which show the specific target side of 4.2.1.20 (Figure 2).

**Table 2.** Bioactive compounds binding interaction with specific protein *hsp-70*

| Ligand (Bioactive compound) | Binding score (Kcal/mol) | Hydrogen bond interaction with key aminoacid residues |
|---|---|---|
| γ-Aminobutyric acid | 24.20 | - |
| α-Tocopherol | 34.67 | ASP 172 |
| Arachidonic acid | 50.21 | TYR 167 |
| Retinol | 34.47 | ASP 172 and SER 166. |
| Docosahexaenoic acid | 49.18 | ASP 172 |
| Phenothrin | 41.36 | - |
| Phylloquinone | 42.05 | - |
| β-Carotene | 32.37 | - |
| Stigmasterol | 31.69 | ASP 172 |
| α-Linolenic acid | 43.81 | ASP172 |
| Astaxanthin | 24.83 | - |
| Brassicasterol | 27.90 | - |
| Ascorbic acid | 24.32 | THR 167, LYS 165 |
| Pyocyanine | 32.14 | - |

This is indicating the final step of tryptophan biosynthesis catalyzed by tryptophan synthase (4.2.1.20). This is the first enzymes known to catalyze two different reactions in two separate active sites connected to each other via a tunnel on the interior of the protein. This amino acid is involved in the necessary component of the animal diet. Oba et al. (2009) reported based on the *in vivo* model synthesis of luciferin for various biolumineseces reactions by including phenylalanine, tyrosine and tryptophan to marine calanoid copepod, *Metridia pacifica*. This observation is supported by the earlier report of Zhang et al. (2006) suggesting that an increase in anthranilate phosphoribosyltransferase (a key enzyme in tryptophan biosynthesis) level in response to environmental change such as oxidative and heat shock stress (HSP-70) indicates the presence of a metabolic machinery constantly sustaining life functions. Previous report shows, the enhancement of HSP-70 during the quiescent to subitaneous egg stage has been reported in *A. tonsa* (Nilsson et al., 2013). These studies suggest that, the bioactive compound, arachidonic acid from microalgae, *P. cruentum* can provide enhanced egg production, augmented growth, and immune stimulation in copepod culture combating stress factors.

## Conclusion

In the present study, the bioactive compound arachidonic acid from microalgae, *P. cruentum* is showing potential for enhancement of HSP-70 by involving in the main cellular synthesis pathway of phenylalanine, tyrosine and tryptophan biosynthesis with specific target site of tryptophan synthase (4.2.1.20). The inclusion of microalga, *P. cruentum* in the diet of copepod culture could provide enhanced egg production leading to high density culture which in turn promotes marine finfish larval rearing. Further *in vivo* investigations should be carried out on copepods for confirming the egg production with this microalgal diet.

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## Compliance with Ethical Standards

### *Authors' Contributions*

Both authors contributed equally for this research works.

### *Conflict of Interest*

The authors declare that they have no conflict of interest.

### *Ethical Approval*

For this type of study, formal consent is not required.

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