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SHORT COMMUNICATION

Role of heat shock protein influencing bioactive compounds from mangrove tropical estuarine microalgae for enhancement of copepod egg production in culture system

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ABSTRACT

In silico investigations of the natural bioactive compounds in the microalgae from mangrove tropical estuaries showed an influence on heat shock protein -70 production. Incorporation of algae with such compounds in the diet of copepod high density culture might lead to enhanced egg production. For this study, the structure of the ligands (bioactive compounds from microalgae in the region of the mangrove estuary) and X-ray crystal structure of hsp-70 complex was taken from PDB (3P9Y) with a resolution of 2.10 Å. The molecular docking study was performed using GOLD software. In the present study, a total of ten bioactive compounds showed good molecular interaction with hsp-70 protein. Among these bioactive compounds, Quercetin from the microalga, *Chlamydomonas eugametos* exhibited the highest molecular interaction and this compound is potential for enhancement of hsp-70 protein compared to other bioactive compounds and is considered a good nutrient enrichment for copepod culture as well as enhancement of hsp-70 protein against ROS and adverse environmental conditions. Successful high density copepod culture might lead to scaling up of hatchery rearing of marine finfish larvae.

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Introduction

Estuarine mangroves ecosystem plays an important role in biodiversity, energy flow and maintaining functioning food chains with phytoplankton (microalgae) as primary producers (Saifullah et al., 2015). A microalga initiates the marine food chain by serving as food to primary consumers such as zooplankton, shellfish, and finfish (Altaff, 2020). The distribution and abundance of commercially important fish and shellfish and their larvae are dependent on some species of microalgae as their main food source. In aquaculture, microalgae are used as a direct food source for various filter feeding larval stages of marine organisms. They are also used as direct food source in the mass culture of copepods (Altaff & Janakiraman, 2015). Cultivation of microalgae is mandatory in the hatchery as it is a basic and nutritious diet for live feed organisms, specifically for copepods. Copepods are of a great ecological importance, being ideal food for larvae of many species of fish. The marine copepods are considered most suitable for the economically valuable cultivable finfish species, as they are a valuable source of protein, lipid (especially HUFA, 20:5 n-3 and 22:6 n-3), enzymes (amylase, protease, exonuclease and esterase), which are essential for larval survival, growth, digestion and metamorphosis (Aman & Altaff, 2004).

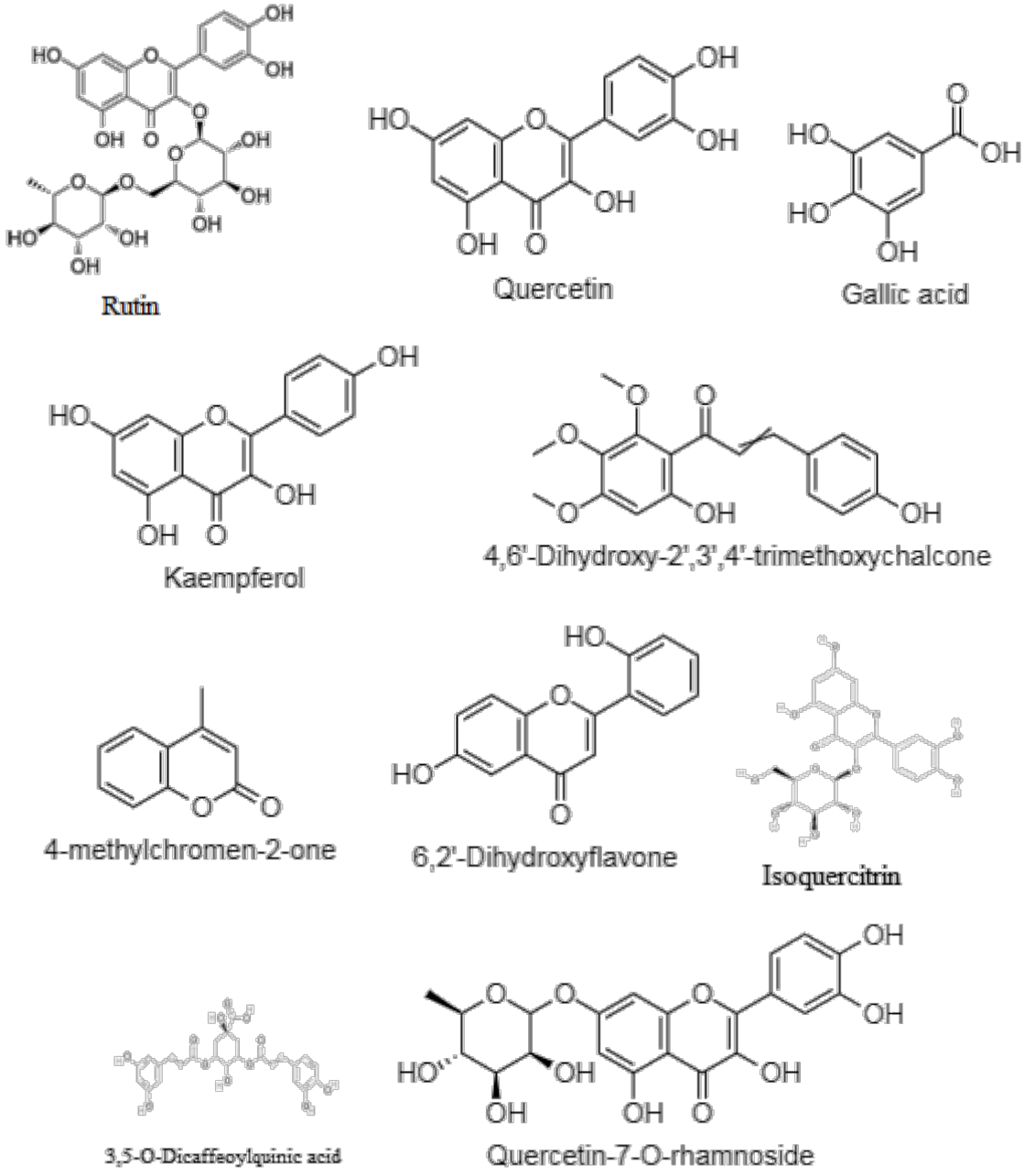

Figure 1. Structure of the ligands of bioactive compounds from microalgae

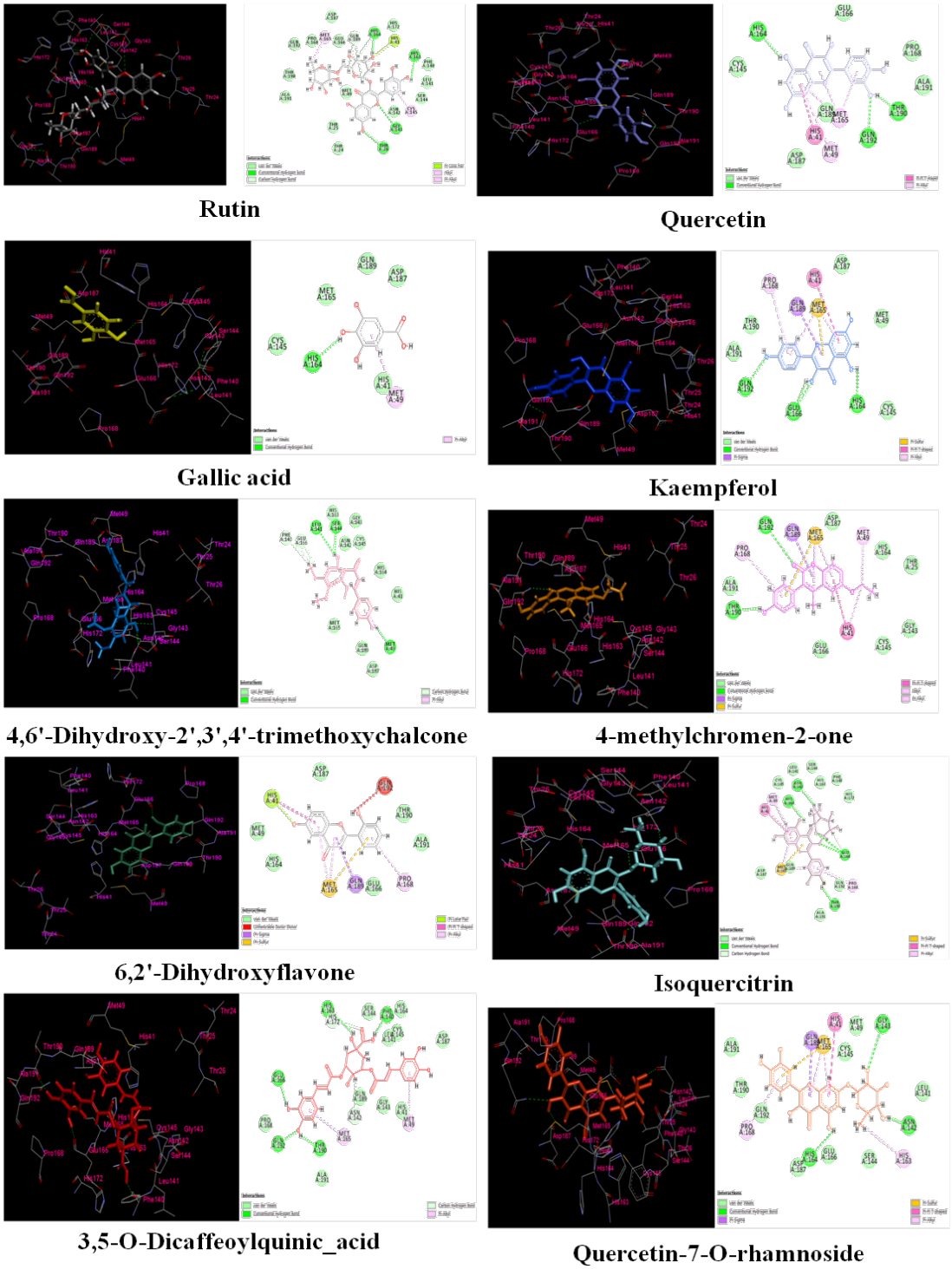

Figure 2. Molecular Interaction of Bioactive compounds with specific protein hsp-70

Under unfavorable conditions some copepod species can produce thick-shelled dormant eggs or resting eggs and with a wider tolerance to temperature and salinity changes. The induction of heat shock proteins (hsps) is considered as an important protective, eco physiologically adaptive, and genetically conserved response to environmental stress in all organisms. Among the hsps, the heat shock protein 70 (hsp-70) production is the response of copepods in shallow waters to protects them against the adverse environmental conditions such as temperature and pH which otherwise leads to damage the cellular macromolecules through reactive oxygen species (ROS) (Nilsson et al., 2014). Based on the above rational, in the present study an attempt is made to search for hsp -70 enhancement bioactive compounds from microalgae in the region of mangrove tropical estuaries 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.

Table 1. Ligands of bioactive compounds from microalgae and their molecular formula

Name of the Bioactive Compounds	Molecular Formula	Bioactive Compound Sources	Reference
Rutin	$C_{27}H_{30}O_{16}$	*Chlorella vulgaris*	Barkia et al. (2019)
Quercetin	$C_{15}H_{10}O_7$	*Chlamydomonas eugametos*	Birch et al. (1953)
Gallic acid	$C_7H_6O_5$	*Euglena cantabrica*	Jerez-Martel et al. (2017)
Kaempferol	$C_{15}H_{10}O_6$	*Microcystis aeruginosa*	Cao & Li (2018)
4,6'-Dihydroxy-2',3',4'-trimethoxychalcone	$C_{18}H_{18}O_6$	*Nannochloropsis oculata*	Sanjeewa et al. (2016)
4-methylchromen-2-one	$C_{10}H_8O_2$	*Porphyridium purpureum*	Su et al. (2016)
6,2'-Dihydroxyflavone	$C_{15}H_{10}O_4$	*Dunalliela* sp.	Lv et al. (2014)
Isoquercitrin	$C_{21}H_{20}O_{12}$	*Schizochytrium aggregatum*	Lv et al. (2014)
3,5-O-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	*Dunaliella salina*	Rismani & Shariati (2017)
Quercetin-7-O-rhamnoside	$C_{21}H_{20}O_{11}$	N*anno chloropsis*	Saifullah et al. (2015)

Material and Methods

The bioactive compounds (ligands) of Rutin from *Chlorella vulgaris*, Quercetin from *Chlamydomonas eugametos,* Gallic acid from Cyanobacteria *Euglena cantabrica*, Kaempferol from Cyanobacterium *Microcystis aeruginosa*, 4,6'-Dihydroxy-2',3',4'-trimethoxychalcone from *Nannochloropsis oculata*, 4-methylchromen-2-one from *Porphyridium purpureum*, 6,2'-Dihydroxyflavone from *Dunalliela* sp., Isoquercitrin from *Schizochytrium aggregatum*, 3,5-O-Dicaffeoylquinic acid from *Dunaliella salina*, and Quercetin-7-O-rhamnoside from N*annochloropsis* sp. were selected for this study (Figure 1). The details of the ligands of bioactive compounds are presented in Table 1. These bioactive compounds occur commonly in the microalgae of mangrove tropical estuaries (Saifullah et al., 2015). For this study, the structure of ligand (bioactive compounds) and X-ray crystal structure of hsp-70 complex was taken from PDB (3P9Y) were received from the databases of PubChem and Protein Data Bank (PDB), respectively. Molecular Docking was performed by GOLD software and detailed methodologies are given in our earlier publication (Altaff & Vijayaraj, 2021).

Results and Discussion

In aquaculture, microalgae constitute direct food source for the larval stages and adults of various filter feeding organisms (Altaff, 2020). They are also used as a direct food source in the production of rotifers, Artemia and copepods which in turn are used as food for the carnivorous larvae of many marine fish species. The microalgae are rich in several chemical compounds such as amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones, alkenes and cyclic polysulphides and are used in several biological applications (Skjanes et al., 2013; Altaff, 2020). Likewise, the microalgae are one of the largest producers of biomass in the estuarine environments. As primary producers the microalgae form the base of the food chain in the estuary and due to the different environmental conditions in the estuarine region, they produce many bioactive compounds which might be considered suitable diet for enhancing copepod reproductive potential in culture system.

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

Ligand (Bioactive compound)	Binding score (Kcal/mol)
Rutin	38.57
Quercetin	46.59
Gallic acid	29.70
Kaempferol	41.67
4,6'-Dihydroxy-2',3',4'-trimethoxychalcone	41.32
4-methylchromen-2-one	44.02
6,2'-Dihydroxyflavone	40.20
Isoquercitrin	46.29
3,5-O-Dicaffeoylquinic_acid	40.40
Quercetin-7-O-rhamnoside	41.72

In recent years, the molecular docking techniques greatly improved the efficiency of research while reduced the cost of research work considerably. It has become a key tool in computer assisted program to predict the binding affinity and analyze the interactive mode of secondary metabolites of bioactive molecules (Vijayaraj et al., 2019, 2021). In the present study, all the bioactive compounds showed good binding interaction with specific protein of hsp-70. The interaction binding energy recorded in the bioactive compounds is presented in Table 2. The molecular interaction of Rutin (38.57 kcal/mol), Quercetin (46.59 kcal/mol), Gallic acid (27.70 kcal/mol), Kaempferol (1.67 kcal/mol), 4,6'-Dihydroxy-2',3',4'-trimethoxychalcone (41.32 kcal/mol), 4-methylchromen-2-one (44.02 kcal/mol), 6,2'-Dihydroxyflavone (40.20 kcal/mol), Isoquercitrin (46.29 kcal/mol), 3,5-O-Dicaffeoylquinic acid (40.40 kcal/mol) and Quercetin-7-O- rhamnoside (41.72 kcal/mol) is depicted in figure-2. Among these bioactive compounds, the bioactive compound, Quercetin from microalga, *C. eugametos* exhibited highest molecular interaction and this compound has higher potential for enhancement of hsp-70 protein compared to other bioactive compounds. *Chlamydomonas* sp. constitute one of the microalgae cultured for use as live feed in aquaculture (Duerr et al., 1998; Sivakumar et al., 2011).

Eggs of many copepods enter in to a resting state, called quiescence, to overcome unfavorable environmental conditions (Sørensen et al., 2007). Under favorable conditions, subitaneous eggs are produced that are characterized by hatching within a few days after spawning (Marcus, 1996). In response to adverse environmental conditions, subitaneous eggs enter a quiescent state, where embryonic development is delayed until exposure to more favorable environmental conditions (Danks, 1987). Quiescence can be defined as a direct inhibition of development due to adverse conditions. Quiescence is a disruption of the embryogenesis that is hypothesized to be associated with transcriptional quiescence and reduced protein synthesis (Hofmann & Hand, 1994; Stuart & Brown, 2006). Thus, the metabolic rate is suppressed during quiescence. Quiescence can be induced in copepod eggs by several adverse conditions (Pedler et al., 1996; Clegg, 1997; Nielsen et al., 2006). Abrupt changes in salinity (Holmstrup et al., 2006), low temperatures (Drillet et al., 2006) and anoxia (Nielsen et al., 2006) have been shown to induce quiescence in order to store copepod eggs over time. When a copepod embryo undergoes quiescence, it requires a number of stabilizing factors (Rhee et al., 2009). Heat shock protein 70 is a family of proteins where some of which are constitutively expressed, while others are induced by several types of stress conditions, such as high and low temperatures, anoxia, reactive oxygen species (ROS), high culture density and osmotic stress. hsp-70 functions as a chaperone molecule that prevents stress-induced misfolding of proteins by facilitating correct folding pathways (Feder & Hofmann, 1999). Under adverse environmental conditions, increased synthesis of hsp-70 is important for the survival of copepods. Aruda et al. (2011) identified several, including four forms of hsp-70 in the calanoid copepod, *Calanus finmarchicus*. hsp-70 has been shown to induce stress alleviation in active *C. finmarchicus* in surface waters, but not in the diapausing animals in deeper waters (Aruda et al., 2011). Voznesensky et al. (2004) and Rhee et al. (2009) found that hsp-70 gene expression is elevated when copepods are exposed to elevated temperatures. The heat shock response of *C. finmarchicus* in shallow waters protects proteins against the higher temperatures experienced under these environmental conditions (Aruda et al., 2011). Changes in available cellular oxygen can result in higher levels of ROS, which in turn causes oxidative stress. ROS includes super oxide ($O2^{-}$), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical (•OH) that is responsible for most damage to cellular macromolecules (Kumaran, 2017, 2018). Because iron is a catalyst in the production of potentially damaging ROS, the amount of free iron in the cell must be minimized to reduce the amount of cellular damage. Quercetin is the most abundant dietary flavonoid found in the natural resources. Quercetin is considered to be a strong antioxidant due to its ability to scavenge free radicals and bind transition metal ions. Quercetin from *Dunaliella tertiolecta* has been reported and these flavonoids acted as a protector of microalgae cells from metal toxicity and unfavorable environment conditions. The extracts containing this compound also showed antioxidant activity (Ferdous et al., 2021). In 1953, Birch et al. described the flavanol quercetin from microalgae *Chlamydomonas eugametos*, where the latter is used as sex hormone. Use of this microalga in the diet of high density culture of calanoid copepods might reduce stress of the copepods as well as enhance their reproductive potential leading to optimize egg production.

To our knowledge there is no published report available on the influence of hsp-70 in the enhancement of copepods egg production. However, only few copepod species have so far been subject in the *in vivo* model among the marine copepods. *Acartia tonsa* response to the heat shock protein 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. Our Docking study indicate use of diet containing Quercetin for copepods in culture system might optimize their egg production by combating adverse culture condition through production of hsp-70.

Conclusion

In the present study the bioactive compound, Quercetin from microalgae, *C. eugametos* is showing highest binding energy and good molecular interaction towards hsp-70 production. Hence the microalgae (*C. eugametos*) *in* the diet of copepod culture might alleviate stress and could provide enhanced egg production leading to high density culture which in turn promote 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

KA: Principal Investigator, Research Supervisor, Experimental Design, Prepared and approved the manuscript.
VR: Performed the experiments, data collection and interpretation, prepared draft manuscript.
MJ: Co-Principal investigator, data analysis and preparation of draft manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

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

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