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Original Article

Prediction of biochemical mechanism of anti-inflammation explained from two marine-derived bioactive compounds

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ABSTRACT

Marine brown macroalgae contain several bioactive compounds with potent anti-inflammatory properties but undescribed pharmacological properties. This study provided the first description of the biochemical mechanism of anti-inflammation from two bioactive compounds (fucoxanthin and sargachromenol) found in brown macroalgae (*Sargassum* spp.) based on a functional module-based analysis of a protein-protein interaction (PPI) network. The constructed PPI network of fucoxanthin and sargachromenol with 18 and 5 inflammatory proteins, respectively, have scale-free, small world and modular properties. There were 6 and 1 inflammatory modules found associated with the anti-inflammatory actions of fucoxanthin and sargachromenol, respectively. Of particular interest was that the anti-inflammatory effect of fucoxanthin and sargachromenol may be partly attributable to regulation of the I-kappa B kinase/NF-kappa B cascade and regulation of gene expression, respectively. These can be used to search for potential targets of fucoxanthin and of sargachromenol to treat inflammation. Therefore, functional module-based analysis of a PPI network can be an initial method for elucidating the anti-inflammatory mechanism of active compounds and finding their targets to validate in a wet laboratory clinical application and for further drug development.

Introduction

Inflammation is a protective response to stimuli that cause tissue injury (Fernando et al., 2016), and activates macrophage cells that produce inflammatory mediators such as nitric oxide (NO), prostaglandins (PG) and cytokines (Lee et al., 2009). Thus, the inhibition of inflammatory mediators is important in inflammation treatment and effective derivatives should be based on action targets (Ham et al., 2015).

Marine brown macroalgae, as a relatively unexplored source of anti-inflammatory agents (Simpi et al., 2013) are distributed worldwide, from temperate to tropical regions including the Gulf of Thailand and Andaman Sea (Yangthong et al., 2009). The genus *Sargassum* (Order Fucales) contains species with potent anti-inflammatory compounds including fucoxanthin and sargachromenol (Yende et al., 2014). Natural compounds that inhibit the production of inflammatory mediators are usually less toxic (Ham et al., 2015). Although anti-inflammatory effects of fucoxanthin and sargachromenol have been shown (Heo et al., 2012; Yang et al., 2013), additional studies at the molecular level would enhance comprehension.

Biological networks represent the collaboration of interactions and forms of processes within a cell, such as protein-protein interactions (PPI), gene regulatory or metabolic networks, that allow for studies of complex cellular processes that result from the interaction and behavior of biomolecules (Faisal et al., 2015). PPI networks are pivotal to the proper functioning of basic biochemical mechanisms within cells, which involve transcription, translation, cell-cell adhesion, communication, synthesis and degradation of the protein, cell cycle control and cellular signaling pathways (Kuzmanov and Emili, 2013). Biochemical mechanism studies based on the PPI of anti-inflammation can be conducted with interaction data between active compounds and proteins that are collected from online databases such as ChEMBL (Davies et al., 2015) and STITCH (Szklarczyk et al., 2016). The experimental and predicted PPI data for a wide variety of organisms can be achieved from an online database such as STRING (Szklarczyk et al., 2017). PPI

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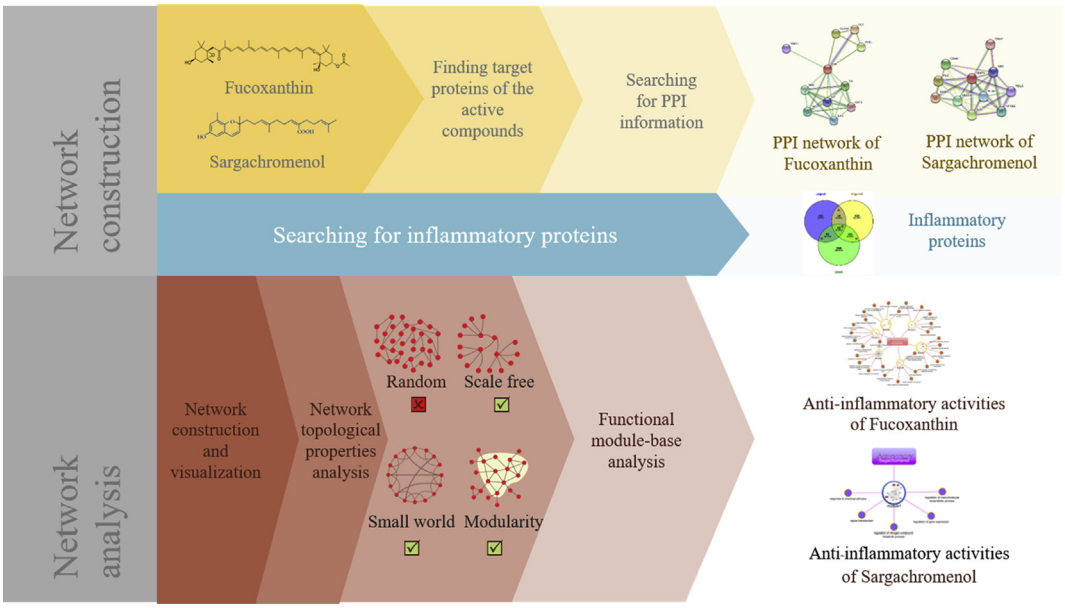


Fig. 1. Overall experiment.

networks, biochemical networks, transcriptional regulation networks, signal transduction or metabolic networks are networked categories that focus on biological systems, which often have common characteristics and properties. This network profiling integrated with knowledge extraction will help to better understand the biochemical mechanism of biological systems (Pavlopoulos et al., 2011).

Several programs are available to visualize and analyze biological networks, including Cytoscape, MCODE and BiNGO. Cytoscape is a widely used open source software platform for visualizing molecular interaction networks and integrating these networks with annotations, gene expression profiles and other state data (Shannon et al., 2003). MCODE is a plugin of Cytoscape for finding clusters in a protein-protein interaction network that are often protein complexes and parts of pathways, while clusters in a protein similarity network represent protein families (Bader and Hogue, 2003). BiNGO is a tool for determining the type of gene ontology (GO) that exists in a set of genes or subgraphs of biological networks (Maere et al., 2005). The GO project provides a set of hierarchical controlled vocabulary split into three categories: biological process, molecular function and cellular component. GO enrichment is widely used for identification-shared associations between proteins and their annotations (Ashburner et al., 2000; Antonazzo et al., 2017). Functional module-based analysis of PPI networks may provide an efficient way to demonstrate the biochemical mechanism of anti-inflammation for several bioactive compounds such as curcumin (Gan et al., 2015), tanshinon IIA (Zheng et al., 2014), salvianolic acid B (Ren et al., 2014) and the active compound contained in the Qishen Yiqi formula (Zheng et al., 2015). However, there is a lack of study that can be applied to explain the appropriate interpretation to then clarify and more effectively explain the biochemical mechanism of anti-inflammation.

In this study, the biochemical mechanism of anti-inflammation from marine-derived active compounds (fucoxanthin and sargachromenol) were examined along with their protein targets for further drug development. PPI networks were constructed using Cytoscape, and the properties of networks were analyzed based on topological parameters. Inflammatory modules of PPI networks were identified and enriched with GO based on protein complex detection.

Materials and methods

Target proteins of the active compounds

Active compounds from brown macroalgae to identify their target proteins were located from the literature related to the anti-inflammatory effect. Fucoxanthin and sargachromenol were selected. Target information on these two active compounds was obtained from ChEMBL (https://www.ebi.ac.uk/chembl) and STITCH (http://stitch.embl.de). ChEMBLis a manually curated database containing binding, functional and ADMET (absorption, distribution, metabolism, and excretion-toxicity in pharmacokinetics) information for a huge range of drug-like bioactive compounds. These data are manually abstracted from the primary published literature on a regular basis, then further curated and standardized. STITCH is an online database of chemical-protein interactions that integrates as much as possible the publicly available knowledge on experimental and manually-curated association information. These target proteins of two selected marine-derived bioactive compounds were searched only in *Homo sapiens*. Their protein symbol and amino acid sequences store data were used to find out the PPI information of target proteins.

Searching for protein-protein interaction information of target proteins

The PPI information of the amino acid sequence of each target protein found in fucoxanthin and sargachromenol was obtained from the online database of STRING 10.0 (https://string-db.org/). The STRING database is intended to collect and integrate information by gathering known and predicted PPI data for a large number of organisms. *Homo sapiens* was the selected organism in this study. The PPI information for all target proteins of bioactive compounds, such as fucoxanthin and sargachromenol, with a confidence score above 0.7 were selected to construct the PPI network using the open-source Cytoscape software (http://cytoscape.org/). Cytoscape provides basic functionality to visually integrate the network with any type of attribute data and to layout and query the network.

(A)

Fucoxanthin		
Target	**UniProt ID**	**Source**
GMNN	O75496	chEMBL
NFE2L2*	Q16236	chEMBL
MAPT	P10636	chEMBL
SMAD3	P84022	chEMBL
ATXN2	Q99700	chEMBL
RECQL	P46063	chEMBL
GLA	P06280	chEMBL
PTH1R	Q03431	chEMBL
ADRB2*	P07550	chEMBL
TP53*	P04637	chEMBL
VDR*	P11473	chEMBL
EHMT2	Q96KQ7	chEMBL
KDM4E	B2RXH2	chEMBL
PCSK9	Q8NBP7	STITCH
TYR	P14679	STITCH
CYP3A4	P08684	STITCH
NR1I3	Q14994	STITCH
CYP1A1	P04798	STITCH
HMOX1*	P09601	STITCH
BCL2L1	Q07817	STITCH
PPARG*	P37231	STITCH
GADD45A	P24522	STITCH
UCP1	P25874	STITCH

(B)

Sargachromenol		
Target	**UniProt ID**	**Source**
AR	P10275	chEMBL
NR3C2	P08235	chEMBL
NR3C1*	P04150	chEMBL
NR1H4*	Q96RI1	chEMBL
RARA	P10276	chEMBL
ESR1	P03372	chEMBL
PGR	P06401	chEMBL

(C)

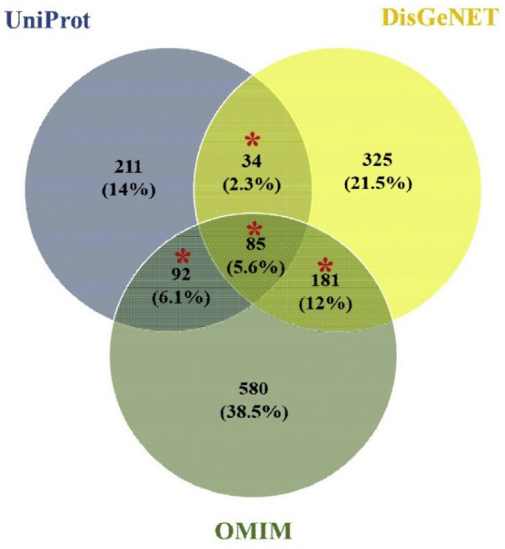


Fig. 2. Target and inflammatory proteins of fucoxanthin and sargachromenol: (A) list of target and inflammatory proteins of fucoxanthin; (B) list of target and inflammatory proteins of sargachromenol; (C) inflammatory proteins common to two of the three databases DisGeNET (Piñero et al., 2015), OMIM (McKusick, 2007) and UniProt (Apweiler et al., 2004), where red star (*) indicates an inflammatory protein.

Searching for inflammatory proteins

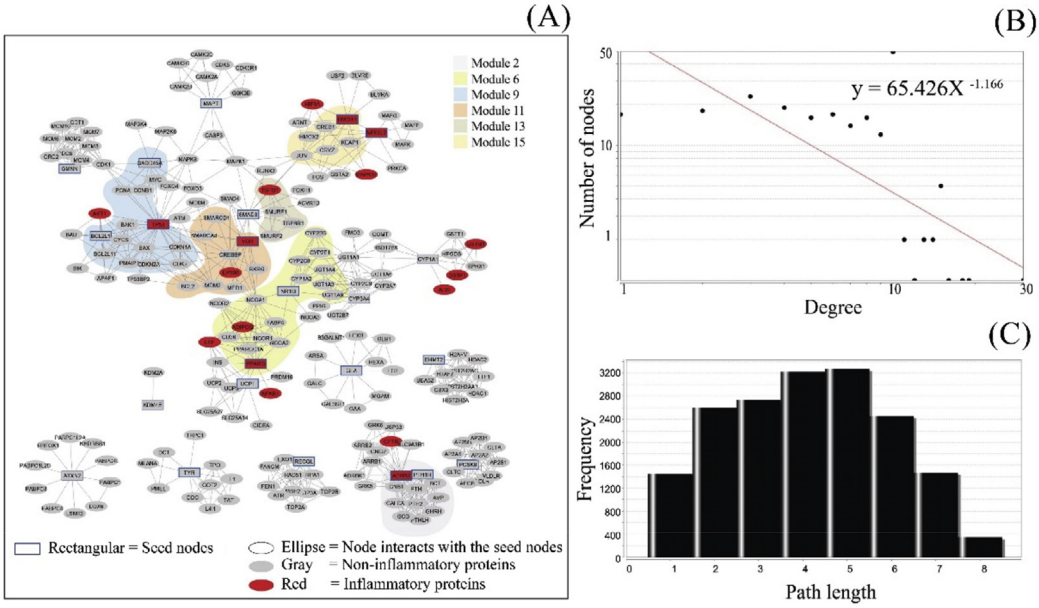
The three online gene/protein-disease databases of DisGeNET (Piñero et al., 2015), OMIM (McKusick, 2007) and UniProt (Apweiler et al., 2004), were used to search for inflammatory proteins. The term "inflammation" was used as a keyword and *Homo sapiens* was the organism selected in this study. Inflammatory proteins were defined as the proteins that can be found associated with “inflammation” in two of the three databases. The Venny program (Oliveros, 2007) was applied to search for inflammatory proteins. These sets of inflammatory proteins were used to analyze target proteins associated with inflammation.

Network topological properties analysis

PPI information of all target proteins in fucoxanthin and sargachromenol was constructed from their PPI networks using the Cytoscape software. Network Analyzer (Assenov et al., 2008), a plug-in of Cytoscape, was employed to analyze topological properties based on parameters, including the clustering coefficient, connected component, network diameter, network centralization, shortest path, characteristic path length and network heterogeneity. Compared with the random network, properties of scale-free, small world and modularity properties of these PPI networks were also investigated.

Functional module-based analysis

The PPI networks were further divided into modules using MCODE, a plugin of Cytoscape, using a cutoff value for the connectivity degree of nodes (proteins in the network) equal to three. The MCODE algorithm finds clusters and highly interconnected regions in a network using graph clustering methods which are relevant for protein networks and are often protein complexes or represent protein families. Inflammatory modules that contained inflammatory proteins were analyzed using GO functional annotation and enrichment using the BiNGO plugin in the Cytoscape software. The overall experiment is shown in Fig. 1.



(D)

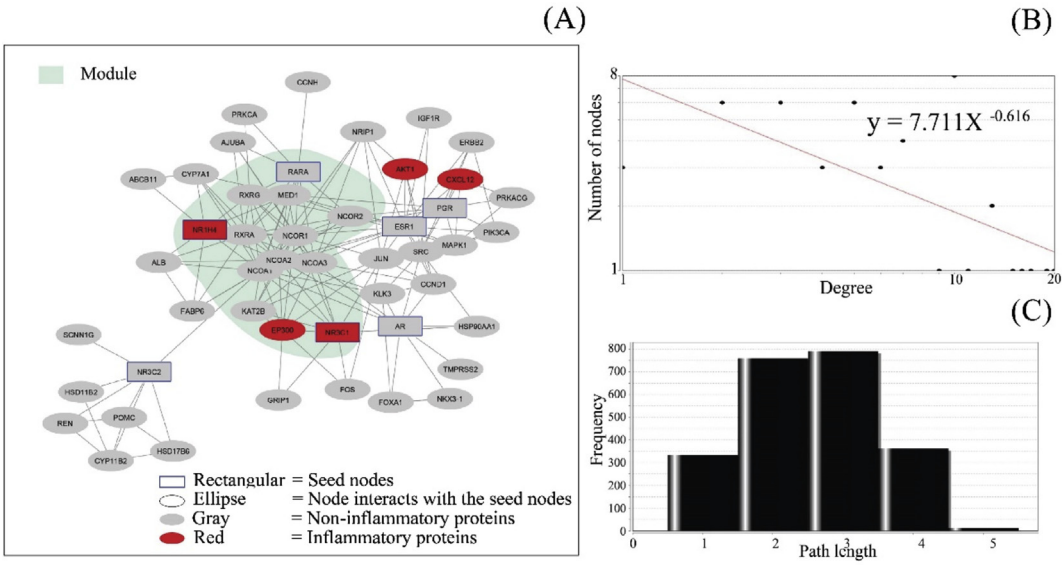
Parameter	Network	
	Protein interaction networks of fucoxanthin	Random Network
Clustering coefficient	0.777	0.034
Connected component	9	1
Network diameter	8	5
Network centralization	0.109	0.034
Shortest path	17554 (37%)	46872 (100%)
Characteristic path length	4.097	3.034
Network heterogeneity	0.608	0.364

Fig. 3. Protein-protein interaction (PPI) network of fucoxanthin and its topological properties. (A) The PPI network of fucoxanthin; (B) Degree distribution of the fucoxanthin network; (C) Shortest path length distribution of the fucoxanthin network; (D) The topological parameters of the PPI network of fucoxanthin. The rectangular nodes present target proteins; The ellipse nodes present proteins interaction with target proteins; The red nodes present inflammatory proteins and the gray ones are nodes that are non-inflammatory proteins.

Results and discussion

The PPI networks of fucoxanthin and sargachromenol, were constructed from their target proteins and used to describe their anti-inflammation activities at the molecular level. Target proteins of both bioactive compounds were obtained from the two online databases ChEMBL and STITCH (Szklarczyk et al., 2016). The target proteins of fucoxanthin and sargachromenol contain 23 and 7 proteins, respectively, and were used to search for PPI information from the STRING online database. Inflammatory proteins were those associated with “inflammation” in two of the three online databases DisGeNET OMIM and UniProt. In total, 392 inflammatory proteins were found and applied to identify target proteins associated with inflammation (Fig. 2C). Six inflammatory proteins were found in the target proteins of fucoxanthin, consisting of NFE2L2, ADRB2, TP53, VDR, HMOX1 and PPARG. Two inflammatory proteins were found in the target proteins of sargachromenol, consisting of NR3C1 and NR1H4. The PPI information of all target proteins of fucoxanthin and sargachromenol were integrated, visualized and their biological network analyzed using Cytoscape. Duplicated interactions of PPI networks were removed using the Advanced Network Merge Plugins in Cytoscape. The constructed PPI network of fucoxanthin contained 217 nodes (proteins) with 18 inflammatory proteins and 725 interactions (Fig. 3A). The sargachromenol network contained 48 nodes with 5 inflammatory proteins and 167 interactions (Fig. 4A).

The fucoxanthin and sargachromenol networks were analyzed for topological properties using Network Analyzer in the Cytoscape software. Topological properties based on topological parameters (clustering coefficient, connected component, network diameter, network centralization, shortest path, characteristic path length and network heterogeneity) in the fucoxanthin and sarcachromenol networks are shown in Figs. 3B and 4B, respectively. The average density of a node neighborhood found in the fucoxanthin and sargachromenol networks (0.777 and 0.651, respectively) is referred to as a clustering coefficient. The network has modularity properties if its clustering coefficient is higher than for a random network. The degree distribution graph at the distribution level was calculated by counting the number of connections between nodes (proteins) of PPI networks. The equations for the degree distribution graphs of the fucoxanthin and sargachromenol networks were $y = 65.426X^{-1.166}$ and $y = 7.711X^{-0.616}$, respectively



Parameter	Network	
	Protein interaction networks of sargachromenol	Random Network
Clustering coefficient	0.651	0.165
Connected component	1	1
Network diameter	5	4
Network centralization	0.29	0.068
Shortest path	2256 (100%)	2256 (100%)
Characteristic path length	2.539	2.198
Network heterogeneity	0.697	0.269

Fig. 4. Protein-protein interaction (PPI) network of sargachromenol and its topological properties; (A) The PPI network of sargachromenol; (B) Degree distribution of the sargachromenol network; (C) Shortest path length distribution of the sargachromenol network; (D) The topological parameters of PPI network of sargachromenol, where rectangular nodes present target proteins, ellipse nodes present protein interaction with target proteins, red nodes present inflammatory proteins and gray nodes indicate non-inflammatory proteins.

(Figs. 3B and 4B). These equations have a scale-free property, due to the equations of the network having a degree distribution that follows a power law distribution $P(k) \sim k^{-\gamma}$ ($\gamma < 3$). The shortest path length distribution (Figs. 3C and 4C) showed that the network path length of the fucoxanthin and sargachromenol networks was mostly concentrated in steps 2–5 and steps 2–3, respectively. The characteristic path length (Figs. 3D and 4D) being the shortest path length between any two proteins in the fucoxanthin and sargachromenol networks was 4.097 and 2.539, respectively, which indicated that most proteins in the network were closely linked and the PPI networks of fucoxanthin and sargachromenol had a small world property. The identified network topological properties suggested the PPI networks of fucoxanthin and sargachromenol had a scale-free property, a small world property and modular properties (Zheng et al., 2015), which showed that the properties of the network could be used in functional module-based analysis.

The fucoxanthin and sargachromenol networks were analyzed from their functional modules using the MCODE and BiNGO algorithms based on the Cytoscape program. Functional module-based analysis of the fucoxanthin network identified 17 modules with 6 inflammatory modules that were interpreted with GO in the biological process that is pathways and larger processes made up of the activities of multiple gene products. These six modules (2, 6, 9, 11, 13, 15) are shown with their GO biological process terms in Fig. 5. For sargachromenol networks, three modules with one inflammatory module were identified (Fig. 6).

The results of inflammatory modules of fucoxanthin show several biological processes consistent with anti-inflammatory responses, including that to stimuli in modules 6, 9 and 11, cytokine production processes found in modules 6 and 13 and signaling an inflammatory response through pathways found in modules 2, 9, 11, 13 and 15. Fucoxanthin can inhibit the degradation of IκB-α and the nuclear translocation of p65 and p50 proteins, resulting in the activation of the NF-κB expression being decreased, which is associated with inhibition of the NF-κB signaling pathway that affects the production of some inflammatory mediators. In addition, fucoxanthin inhibits the phosphorylation of ERK1/2, JNK and p38 proteins in MAPKs signaling pathways. In addition, p38, activated by LPS, controls iNOS, COX-2 and TNF-α gene expression. ERK is involved in LPS-induced macrophage responses and increases in the production of inflammatory mediators and iNOS. Furthermore, JNK also regulates the expression of LPS-induced iNOS (Kim et al., 2010b). Fucoxanthin inhibited protein and mRNA expression of iNOS and COX-2, the inhibition of NO and PGE_2 production. PGE_2 is an important mediator in the inflammatory process produced by COX-2. NO is an inflammatory mediator produced by iNOS, by

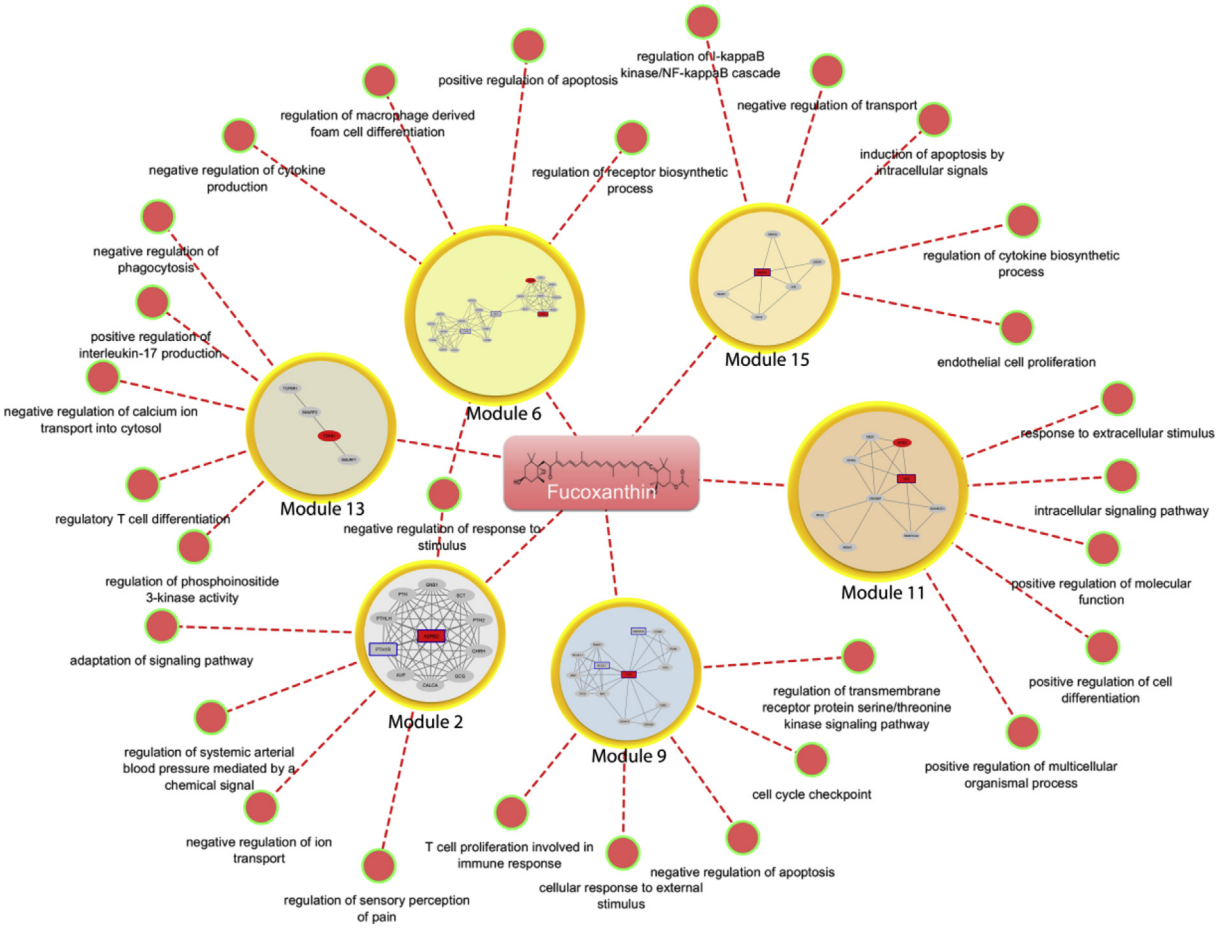


Fig. 5. Anti-inflammatory activities of inflammatory modules in fucoxanthin network.

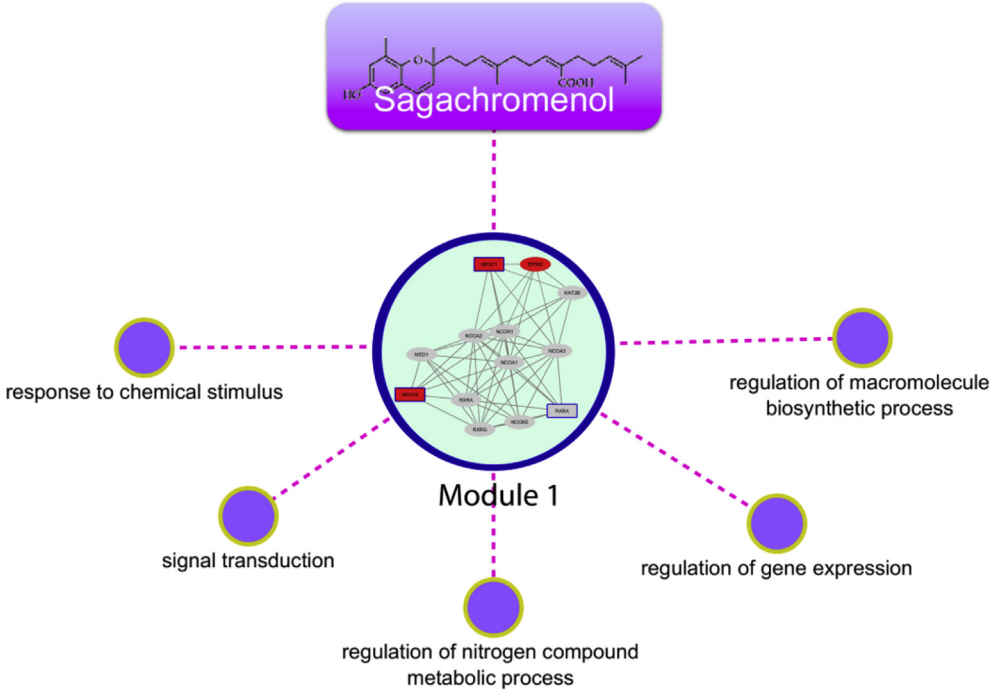


Fig. 6. Anti-inflammatory activities of inflammatory modules in sargachromenol network.

which overproduction of NO and PGE_2 can lead to inflammation, cytotoxicity and inflammatory disease (Heo et al., 2012). Fucoxanthin also inhibits production of various cytokines such as TNF-α, IL-6, and IL-1β, which play an important role in inflammation processes (Heo et al., 2010). TNF-α is a macrophase activator that can stimulate the production or expression of IL-6, IL-1β, PGE_2, collagenase and an adhesion molecule (Brennan and McInnes, 2008). IL-6 is a cytokine and is an intracellular inflammatory mediator induced by LPS that causes fever (Evans et al., 2015). IL-1β is a cytokine released by macrophages and plays an important role in the pathophysiology of rheumatoid arthritis (Heo et al., 2010). Fucoxanthin has been reported to inhibit the production of inflammatory mediators such as NO, PGE2, TNF-α, IL-1β, and IL-6 by the suppression of phosphorylation in the NF-κB and MAPK signaling pathways (Zhang et al., 2015).

Fucoxanthin is involved in ion transport described in modules 2, 13 and 15 that are important in inflammatory processes (Eisenhut, 2006; Eisenhut and Wallace, 2011). Changes in ion transport affect inflammatory processes associated with allergies, auto-immune diseases, and infection (Eisenhut and Wallace, 2011). Inflammatory mediators such as NO and PGE_2, are associated with down regulation of sodium transport affecting both protein levels and gene expression of ENaC and Na/K ATPase (Eisenhut, 2006). PGE_2 inhibits ENaC in the cell membrane by induction thereby reducing the activity or expression of the Na/K ATPase at the basolateral membrane. PGE_2 also stimulates chloride and inhibits sodium transport, respectively. All tissue systems have a PAR2 receptor involved in regulation of Cl-transport and signaling inflammatory mediators (Eisenhut, 2006). The ion transport processes inhibiting inflammation have not been described for fucoxanthin. Fucoxanthin induces and regulates apoptosis, which is related to modules 2, 6, 9, and 15, with the former being a selective process of physiological cell elimination. Fucoxanthin regulates the balance between cell proliferation and cell death involved in the Bcl-xL signaling pathway. Fucoxanthin regulates the Bcl-xL signaling pathway for reactive oxygen species production by reducing the level of Bcl-xL expression, activation of Caspase-3 and Caspase-7, and PARP cleavage. Bcl-xL is an upstream molecule in the apoptotic pathway and is a potent suppressor of apoptosis (Kim et al., 2010a) (Fig. 5). Ion transport processes are involved in gene and protein expression of ENaC and Na/K ATPase as in the regulation of apoptosis involved in the Bcl-xL signaling pathway. Current understanding of both processes is incomplete. Therefore, the results of this study, and the further prediction of the roles of the active compound may impact on its further analysis in the laboratory.

Module 1 of the sargachromenol network is associated with response to chemical stimulus, signal transduction and regulation of gene expression that are more related to inflammatory response (Fig. 6). Sargachromenol is related to inflammation by reducing the expression of iNOS and COX-2 proteins that can reduce the production of NO and PGE_2. Both are inflammatory mediators in inflammatory disease mechanisms. Sagachromenol may provide its anti-inflammatory effects on LPS-stimulated macrophage cells by decreasing the cytoplasmic loss of the inhibitor κBα (IκBα) protein that inhibits the activation of NF-κB signaling pathways (Yang et al., 2013). Although studies are incomplete, sargachromenol may be tested with the other signaling pathways such as MAPK that regulate inflammation in gene expression of several inflammatory mediators.

In summary PPI networks from bioactive fucoxanthin and sargachromenol present in marine brown macroalgae were examined for their inflammatory mechanisms using functional module-based analysis. Public databases and tools were applied for PPI network construction and analysis. The fucoxanthin network contained 217 nodes with 18 inflammatory proteins and 725 interactions and the sargachromenol network had 48 nodes with 5 inflammatory proteins and 167 interactions. The two networks possessed the topological properties of scale-free, small world and modularity, which are important features in functional module-based analyses. Inflammatory proteins were those associated with “inflammation” in two of the three online databases. The functional module-based analysis identified 17 modules with 6 inflammatory modules from the fucoxanthin network. The sargachromenol network contained three modules with one inflammatory module. The inflammatory modules of fucoxanthin and sargachromenol networks were interpreted to involve many inflammatory processes. This study demonstrated the biochemical mechanism of anti-inflammation from fucoxanthin and sargachromenol may be partly attributable to regulation of the I-kappa B kinase/NF-kappaB cascade and regulation of gene expression, respectively. Therefore, the functional module-based analysis of the PPI network and its biological interpretation are basic to understanding the role of fucoxanthin and sargachromenol at the computer level and in providing important guidelines to validate its clinical application and further drug development.

Conflict of interest

None.

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