Nutraceutical products from seaweeds - wonder herbs of the oceans

Kajal Chakraborty*, P. Vijayagopal and A. Gopalakrishnan
ICAR-Central Marine Fisheries Research Institute, Cochin - 682 018, Kerala, India

*E-mail: kajal.chakraborty@icar.gov.in

Abstract

Seaweeds encompassing several species are common in the coastal areas of Indian subcontinent, and can be termed as the wonder herbs of the ocean due to their potential pharmaceutical properties. Marine-based resources are drawing the attention of nutraceutical industries due to their protective function against various chronic diseases and the growing demand for new compounds of 'marine natural' origin. Considering this, ICAR-Central Marine Fisheries Research Institute developed research program to systematically search identified seaweed species for the development of promising bioactive molecules. The nutraceutical products Cadalmin™ Green Algal extract and Cadalmin™ Antidiabetic extract as green alternatives to synthetic drugs to combat rheumatic arthritic pains and type-2 diabetes, respectively were developed. Following this, Cadalmin™ Antihypercholesterolemic extract and Cadalmin™ Antihypothyroidism extract to combat dyslipidemia and hypothyroid disorder, respectively were also developed. The lead molecules with action against angiotensin converting enzyme-I, from seaweeds were also isolated, and added to a nutraceutical product Cadalmin™ Antihypertensive extract that is being out-licensed.

Keywords: *Seaweeds, secondary bioactive metabolites, nutraceutical products, Cadalmin™*

Introduction

The oceans cover about 70% of the planet's surface with a high biodiversity. Yet, very few marine species have been explored or used for pharmaceutical purposes. The bioactive metabolites of marine origin have attracted the attention of medical practitioners and scientists for the past few decades because several of these chemicals exhibit pronounced pharmacological activities. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their nutrient, pigments, and chemical composition and formed part of the traditional diet of coastal communities particularly in Japan, China and Korea. The discovery of metabolites with biological activity from seaweeds has increased substantially in the last three decades. These substances exhibit an appreciable number of distinct biological activities, such as anti-tumoral, anti-viral, anti-fungal, insecticidal, cytotoxic, phytotoxic, and anti-proliferative actions. Most of the bioactive substances isolated from seaweeds are chemically classified as brominated, aromatics, nitrogen-heterocyclic, nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides, sulphated polysaccharides, terpenes, acetogenins, alkaloids and polyphenolics. Seaweeds are also the only sources for industrially important phycocolloids like agar, carrageenan and alginate which have applications as stabilizer, viscosifier, gelling and emulsifying agents.

Why seaweeds are prolific producer of pharmacologically active metabolites

Seaweeds were reported to possess structurally diverse compounds of various bioactivities endowed with antihypertensive, anti-inflammatory, and anticarcinogenic activities. These species which grows in saline habitats

Fig.1. Cultured seaweed harvest at Mandapam, Tamil Nadu

Fig.2. Seaweed harvest from Indian seas

evolved a number of specialized biochemical mechanisms to withstand salt-triggered oxidative stress conditions, which is governed by multiple biochemical mechanisms facilitating cell homeostasis and ability for retention of water. However, the absence of oxidative damage in the structural components, deterrence of predation, and the ability to reproduce successfully suggest that their cells are the storehouse of bioactive metabolites with potential pharmacological properties. Therefore, these marine floras are considered as valuable sources of bioactive compounds with potential pharmacological significance.

Nutraceuticals from seaweeds

The rich diversity of seaweeds represents an untapped reservoir of bioactive compounds with valuable pharmaceutical and biomedical use. The pioneering research work at ICAR-Central Marine Fisheries Research Institute (CMFRI) involved chemical profiling of major species of seaweeds for lead pharmacophores coupled with evaluation of target biological activities against different disease models, for example, 3-hydroxy-3-methylglutaryl coenzyme A reductase, type-2 diabetes modulators (dipeptidyl peptidase-4, protein tyrosine phosphatase 1B), angiotensin converting enzyme, inflammatory cyclooxygenase-2 and 5-lipoxygenase. Optimized physical/chromatographic procedures were developed to isolate and purify the molecules with target bioactivities.

A database of seaweeds with high-value bioactive molecules responsible to combat various life-threatening and lifestyle diseases could be developed. This research could also develop protocols to prepare nutraceutical products enriched with lead pharmacophores with different properties against various drug targets for use against hypothyroidism, dyslipidemia, hypertension, type-2 diabetes, and inflammatory disorders (Fig.3). The natural anti-inflammatory supplements enriched with lead molecules as nutraceutical Cadalmin™ Green Algal extract (Cadalmin™ GAe) from seaweeds as effective green alternative to the synthetic drugs available in the market to combat rheumatic arthritic pains was subsequently out-licensed to the biopharmaceutical company for commercial production and marketing in India and abroad. The research efforts to isolate the lead molecules with action against type-2 diabetes led to the development of a nutraceutical product Cadalmin™ Antidiabetic extract (Cadalmin™ ADe). Cadalmin™ Antihypercholesterolemic extract (Cadalmin™ ACe) and Cadalmin™ Antihypothyroidism extract (Cadalmin™ ATe) developed to combat dyslipidemia and hypothyroid disorders, respectively, were also out-licensed to a pharmaceutical company.

What are nutraceuticals and how they are different from drugs?

The term "nutraceutical" was coined by Dr. Stephen DeFelice, founder and chairman of the Foundation for Innovation in Medicine who defined it as "any substance that is a food or a part of a food and provides medical or health benefits, including the prevention and treatment of disease". Nutraceutical has been defined as "concentrated, isolated, or purified" pharmacologically bioactive molecules. Nutraceuticals are clearly not drugs, and unlike synthetic drugs, the potential pharmacologically active substances are derived from natural sources, and are concentrated by using green extraction/purification techniques. The purification process eliminates the unnecessary components in the product, and increases the quantities of the intended pharmacophore(s), which are specifically active against a particular disease. This apparently leads to greater pharmacological activities of the nutraceutical products, while maintaining the mean lethal dose (LD_{50}) greater than the threshold limits indicate the safety of the products. The LD_{50} of the nutraceutical products developed by ICAR-CMFRI were found to be greater than 4000 mg/kg body weight of the mammalian subjects tested that indicated the safety of the products. Since early 2000s, the world has viewed the extensive growth of the billion dollar nutraceutical industry and nutraceuticals are the preferred product portfolio of the leading pharmaceutical companies in India and abroad. The greatest challenge remains to formulate the regulatory guidelines to enable the physicians to prescribe this group of specialized medicines, and this will encourage research and development of this group of products.

Nutraceutical products developed by ICAR-CMFRI

The nutraceutical products Cadalmin™ Green Algal extract (Cadalmin™ GAe) and Antidiabetic extract (Cadalmin™ ADe) as green alternatives to synthetic drugs to combat rheumatic arthritic pains and type-2 diabetes respectively, were developed from seaweeds by ICAR-CMFRI. Cadalmin™ Antihypercholesterolemic extract (Cadalmin™ ACe) and Cadalmin™ Antihypothyroidism extract (Cadalmin™ ATe)

developed to combat dyslipidemia and hypothyroid disorder, respectively were also commercialised. Semi-synthetic C-4/C-6 methylene-polycarboxylate cross-linked hybrid drug delivery system and a topical antibacterial formulation developed from seaweeds were found to be comparable with commercially available products. The lead molecules from seaweeds, with action against angiotensin converting enzyme-I, were isolated and added to a nutraceutical product Cadalmin™ Antihypertensive extract (Cadalmin™ AHe). Seaweed-derived natural template inspired synthetic derivatives as potential pharmacophores with potential antibacterial activities against methicillin-resistant *Staphylococcus aureus* and anti-angiotensin-I inhibitory activities were also designed and developed. Several cosmeceutical products from seaweeds are also in pipeline or being commercialized.

CadalminTM ADe: The bioactive ingredients in Cadalmin™ ADe competitively inhibit dipeptidyl peptidase-4 and tyrosine phosphatase 1B thereby hindering the occurrence of type-2 diabetes. Type-2 diabetes and obesity are characterized by resistance to hormones insulin, possibly due to attenuated or diminished signaling from the receptors. A large body of data have identified protein tyrosine phosphatase 1B (PTP1B) as a major negative regulator of insulin signaling. Pharmacological agents capable of inhibiting the negative regulator(s) of the signaling pathways like PTP 1B are expected to potentiate the action of insulin and therefore be beneficial for the treatment of type 2 diabetes. Antidiabetic extract inhibits PTP1B, thereby hindering the occurrence of type-2 diabetes. Another mode of action of Antidiabetic extract is inhibition of dipeptidyl peptidase-4 (DPP-4), which is an antigenic enzyme expressed on the surface of most cell types and is associated with immune regulation and signal transduction. DPP-4 inactivates the incertins GLP-1 and GIP by removing amino acids from these peptide hormones. GLP-1 and GIP are essentially required for insulin secretion from the βcells of pancreas. *In vitro* antidiabetic experiments showed that the active principles effectively inhibited DPP-IV, tyrosine phosphatase, and βglucosidase. The results demonstrated the potential of the formulation to effectively inhibit the mediators, which are responsible to induce type-2 diabetes through various metabolic pathways. The product developed from seaweed was compared with that of standard drugs after administering the animals with streptozotocin (a diabetes inducer). The diabetic control had glucose level recorded at greater than 380 mg/dL, whereas the blood glucose levels maintained at about 74 mg/dL (at 65 mg/kg body weight), when the animals were administered with the active ingredients. The HbA1c levels maintained at about 4.6% (the normal range being 4.3-6.3%) after administering the animals with the nutraceutical product. The active principles of Cadalmin™ Antidiabetic extract from seaweeds thus effectively inhibit various mediators, which are responsible for inducing type-2 diabetes through various metabolic pathways. The bioactive ingredients in the nutraceutical product interfere with the release of simple sugars from the gut, which in turn reduces postprandial (after eating) hyperglycemia (high blood sugar levels). It has no side effects (LD_{50} > 5000 mg/kg body weight) as proved from the preclinical and acute/long term chronic toxicity studies on experimental subjects. The active ingredients in the product packed in plant-based capsules to meet the dietary needs of vegetarians has a promising consumer appeal, and market potential especially for the large vegetarian population in India and abroad.

CadalminTM GAe: The product is effective for combating arthritic pain and inflammatory diseases in human beings. The active principles in Cadalmin™ GAe competitively inhibit pro-inflammatory mediators, resulting in

Fig. 3. Nutraceuticals developed by ICAR-Central Marine Fisheries Research Institute for use against rheumatoid arthritis, type-2 diabetes,obesity/dyslipidemia,hypothyroidism and hypertension (Left to right)

decreased production of inflammatory prostaglandins and leukotrienes, and its activity was found to be superior to some of the synthetic non steroidal anti-inflammatory drugs available in the market. A lower cycloxygenase-1/5-lipoxygenase and cycloxygenase$_{1/2}$ (<1.0), simultaneous inhibition of cycloxygenase-2 and 5-lipoxygenase enzymes and significant *in vivo* activity indicate higher selectivity and lower side-effect profiles of Cadalmin™ GAe as compared to the synthetic non-steroidal anti-inflammatory drugs. Time dependent *in vivo* animal model studies on experimental subjects revealed the superior inhibition of inflammatory response to the tune of 73-76% by Cadalmin™ GAe. Long term animal model experiments proved the efficiency and safety of this nutraceutical. Cadalmin™ GAe suppresses the edema produced by histamine, and exhibits its anti-inflammatory action by means of either inhibiting the synthesis, release or action of anti-inflammatory mediators. The mean lethal dose (LD_{50}) of Cadalmin™ GAe was found to be greater than 4000 mg/kg body weight of the mammalian subjects that indicate the safety of the product. As part of the preclinical assay of the product, feeding of Cadalmin™ GAe even at a dose upto 2500 mg/kg body weight did not induce significant change in body weights, hematological indices, histopathological, and serum biochemical parameters between the control and treated groups. This product has been commercialized with a biopharmaceutical company and ICAR-CMFRI is in search of more commercial partners for wider dissemination of the product in the marketplace.

CadalminTM ACe: Bioactive pharmacophore leads from seaweeds were used to develop the nutraceutical product, and were found to inhibit hydroxymethyl glutaryl coenzyme A reductase, various target receptors and other rate limiting enzymes, which are responsible to cause obesity and dyslipidemia. The total cholesterol ($\sim$ 90 mg/dl), triglyceride (TG 88.14 mg/dl), high density cholesterol (HDL 39.18 mg/dl), low density cholesterol (LDL 28.09 mg/dl) and very low density cholesterol (VLDL 22.36 mg/dl) were found to be within the acceptable limits after oral administration of the product in the mammalian subjects, with respect to positive control (total cholesterol 185.5 mg/dl, TG 193.28 mg/dl, HDL 33.65 mg/dl, LDL 110.9 mg/dl and VLDL 38.86 mg/dl). *In-vivo* evaluation of the product prevents high fat-induced (HFD) hyperlipidemia, characterized by significantly lower level of serum LDL (28.09 mg/dL), lipid peroxidation status (1.93 nmoles/mg protein) and, substantially lower atherogenic index (0.11 mg/g tissue), constitutes a promising anti-lipidemic efficacy of the formulation. hydroxymethyl glutaryl reductase enzyme inhibitory activity of the formulation was found to be greater than 90% at a concentration of 5 mg/mL. This product showed potential to inhibit hydroxymethyl glutaryl reductase (IC_{50} 0.15 mg/mL), an established statin target, and reduce obesity and hypercholesterolemic disorders as determined by the *in vivo* mammalian model. Other liver and lipid parameters, which clinically signify dyslipidemia and obesity, were also found to be within the clinically acceptable limits. Time dependent shelf life studies conducted to identify the oxidative changes for the product in an accelerated shelf-life study revealed that the content of the bioactive pharmacophores of the product on shelf for a period equivalent to 24 months exists. The product was out-licensed in 2017.

CadalminTM AHe: The active principles of Cadalmin™ AHe from seaweeds effectively inhibit various mediators, which cause hypertension through various metabolic pathways. It blocks angiotensin converting enzyme that converts angiotensin I to angiotensin II. The bioactive ingredients in the nutraceutical product effectively modulate the serum level of oxidative stress marker nitric oxide, lipid peroxidase and the potent vasoconstrictor angiotensin-II which increases blood pressure, and promotes inflammation and remodeling of the cardiovascular system, which leads to thrombosis or ventricular hypertrophy. Animal model anti-hypertension experiments showed that the active principles effectively decreased the angiotensin-II levels in the cadmium chloride ($CdCl_2$) induced hypertension in rats. Serum nitric oxide, lipid peroxidase and angiotensin-II levels were also significantly decreased in hypertension affected group treated by Cadalmin™ AHe. In $CdCl_2$ plus Cadalmin™ AHe group serum NO level has been significantly regulated upto 8.5 μg/dL at 100 mg/kg body weight and 9.00 μg/dL at 200 mg/kg body weight compared to the diseased group (13.06 μg/dL at 100 and 200 mg/kg body weight) and positive control group (9.17 μg/dL at 100 and 200 mg/kg body weight). The serum angiotensin-II level in $CdCl_2$ + Cadalmin™ AHe group were comparatively lesser 0.205 pg/mL at 200 mg/kg body weight than the diseased group (0.432 pg/mL at 200 mg/kg body weight) and positive control (0.211 pg/mL at 200 mg/kg body weight). Preclinical trials showed no toxicity related significant changes in renal or hepatic function, hematological indices and serum biochemical parameters in the experimental subjects. The results also demonstrated a lack of test substance-related general organ or systemic toxicity and hypertensive disorders following oral administration at a dose as high as 2000 mg/kg/d. No side effects ($LD_{50} > 4000$ mg/kg BW) as proved from the acute/

long term chronic toxicity studies on experimental subjects were recorded. This product is available in encapsulated form and is intended to be used as oral application. Large scale extraction of the active principles from the raw material was optimized in a factory unit.

CadalminTM ATe: This nutraceutical with anti-hypo thyroidism principles extracted from seaweed, with an ecofriendly "green" technology has been commercialized by a leading Indian MNC in wellness during the year 2018. The bioactive leads concentrated in Cadalmin™ ATe were found to stimulate thyroid releasing hormone and increase the activity of selenodeiodinase to produce metabolically active thyroid hormones tetraiodothyronine (T_4) and 3, 5, 3'-triiodothyronine (T_3). The current mode of treatment for hypothyroidism is levothyroxine-replacement therapy. However, there are certain limitations associated with this as it requires lifelong treatment, and is associated with poor compliance in some patients. Hence effective and alternative therapeutic strategies to treat hypothyroidism have been sought. The TSH in thyroid gland is responsible for the synthesis, storage and release of metabolic hormones thyroxine (T_4) passive hormone containing four iodine and triiodothyroxine (T_3) active hormone. The predominant hormone produced by the thyroid gland is T_4, with approximately 70-90 mcg of T_4 and 15-30 mcg of T_3 produced daily. The production of the T_3 hormone by the thyroid gland is insufficient to meet the daily requirements of the organs in the body. Therefore, approximately 80% of the body's required T_3 comes from peripheral conversion of T_4 to T_3. Although both T_4 and T_3 are active, T_3 is more active as thyroid receptors within the cell nucleus have a 10-fold greater affinity for T_3. *In vivo* anti-thypothyroidism experiments showed that the active principles effectively increased thyroid stimulating hormone to produce thyroid hormones (T_3 and T_4) into various experimental groups with healthy control and hypothyroidism induced by administering methimazole (MTZ) @100-150 mg/kg body weight (@ one-time dose for 15 days). Serum T_3, T_4 and TSH levels were significantly decreased in hypothyroid group. MTZ is a reversible goitrogen and induces hypothyroidism by inhibiting crucial enzyme deiodinase required for thyroid hormone synthesis. Inhibition of deiodinase impairs the iodination of tyrosyl residues and coupling of iodotyrosyl residues to form iodothyronine. Hypothyroid rats treated with Cadalmin™ ATe exhibited an improved thyroid profile. In MTZ+active ingredient groups with and without bioactive ingredients from seaweed, serum T_3, T_4 and TSH levels were significantly elevated respectively compared to diseased group. The results demonstrated the potential of Cadalmin™ ATe to effectively stimulate the production of thyroid hormones. Cadalmin™ ATe developed from seaweed was compared with that of standard drugs after administering the animals with methimazole (MTZ) (a hypothyroidism inducer). Serum triiodothyronine (T_3) level for the active ingredient with Cadalmin™ ATe treated group (1.4 ng/dL at 150 mg/kg body weight) was greater than the active ingredient alone treated group (0.7 ng/dL at 150 mg/kg body weight) and positive control group (~1 ng/dL). Notably thyroxine (T_4) level for the active ingredient with Cadalmin™ ATe treated group (9 μg/dL at 150 mg/kg body weight) was greater than the active ingredient alone treated group (5 μg/dL at 150 mg/kg body weight) and positive control group (6 μg/dL). One of the bioactive components combines with the tyrosine (aromatic amino acid) to synthesize thyroid hormones to create a more stable, steady supply of iodine for the thyroid. The deiodinase-activativing bioactive component in Cadalmin™ ATe was found to play significant role in the control of thyroid hormone metabolism and hence useful to treat hypothyroidism.

Conclusion

Seaweed derived bioactive components with potential health benefits are an emerging area of research. Seaweeds have a long tradition as a food source in Asian countries, being part of the Western diet only to a limited extent. In 2014, production of seaweeds through mariculture (44% of all aquaculture) was estimated at about 27 million tons wet weight, registering annual growth rate of 8% and valued at 7 billion US$ (FAO 2016). The Regional Centre ICAR-CMFRI at Mandapam, Tamil Nadu ventured to develop indigenous cultivation technology for seaweeds since 1970s. Considering the present status of under-utilization of seaweeds, exploring bioactive compounds and development of any biologically useful products can bring dual benefits-one, as health products and secondly, commercial farming in coastal habitats, resulting in C-sequestration and C-budgeting in a scenario where global climate change poses a serious threat. Development of value-added products from these underutilized species will promote their farming in coastal habitats, which has not been seriously explored earlier due to the lack of knowledge about their commercial importance. Devoted research program to develop various health products from seaweeds will also pave the way to effectively harness the potential of this natural wealth of Indian coastal waters.