



Research Article

PROXIMATE AND PHYSICOCHEMICAL COMPOSITION OF MARINE RED SEAWEED *PORTIERIA HORNEMANNII* AGAINST ANTI DIABETIC ACTIVITY

¹Saranya Karuppannan, ¹Kokila Palani, ¹Nithya Palaniappan, ¹Archana Lakshmanan, ²Viji Maluventhan, and *¹Maruthupandian Arumugam

¹Ethanopharmacological and Algal Biotechnology Laboratory, Department of Botany, School of Life Sciences, Periyar University, Periyar Palkalai Nagar, Salem-11.

²Department of Botany, Thiagarajar College, Madurai – 625009, Tamil Nadu, India.

Article History: Received 26th August 2022; Accepted 22 September 2022; Published 9th October 2022

ABSTRACT

The proximate composition (Protein, carbohydrate, lipid, ash, moisture and total dietary fiber content) and physicochemical properties of *Portieria hornemannii* were studied. The proximate composition of protein 33.82 ± 1.48 followed by carbohydrate and lipid the protein is highest activity. Total dietary fiber contained 2.24 ± 0.39 for the red seaweed of *Portieria hornemannii* contains neutral sugars corresponded to sulphated galactans (Carrageen and agar). Insoluble fiber 1.22 ± 0.06 and soluble fiber 1.02 ± 0.05 , moisture and ash were 12.96 ± 0.55 and 18.42 ± 1.03 respectively. Followed by WHC and swelling both had strong physicochemical characteristics at high temperatures. The oil retention is very low. Finally the seaweed *P.hornemannii* is used in anti diabetic activity of α amylase enzyme activity IC50 value 121.68. The *P.hornemannii* can be effective inhibition of anti diabetic activity and its leads to develop the natural drug in pharmaceutical application.

Keywords: *Portieria hornemannii*, Protein, Physicochemical properties and Anti diabetic activity.

INTRODUCTION

The seaweeds have a various bioactive potential of the worldwide usage of marine products in the treatment of diabetes is boosting patient demand for natural remedies. In past decades seaweeds have largest amount of potential bio active compounds in many industries. Secondary metabolites of seaweed have been isolated in 2400 aquatic environments, and these chemicals are utilized for a multitude of bio-functional applications (Sanger *et al.*, 2019). As a new source of bioactive compounds with significant therapeutic potential, macro algae are utilized in production of large quantities of pharmaceutical for humans (Basheer *et al.*, 2020). In western countries peoples have consuming seaweeds as a part of their food products. The seaweeds are used in many applications in industry such as phycocolloids, food contains thickening and gelling ingredients (Mendes *et al.*, 2022).

Seaweeds that are edible have few calories and a lot of vitamins, unsaturated fatty acids, and dietary fiber that are good for managing diabetes (Khalid *et al.*, 2022). With their reserve of starch-digesting enzymes, seaweed bioactive compounds play a significant role in the reversal of glucose-stimulated oxidative stress. Few previous investigations have discovered antidiabetic effects in diverse marine algae, making them one of the less researched sources of pharmacological possibilities. Micro and macronutrients such as, carbohydrates protein, vitamins, minerals, and polyphenols are abundant in marine seaweed (El-Beltagi *et al.*, 2022). In general, seaweeds are quite nutritious. One of the disorders characterized by chronic hyperglycemia due to a deficiency in insulin secretion or resistance is diabetes mellitus (Unnikrishnan *et al.*, 2015, Nickavar and Yousefian, 2011). The currently used therapeutic approach involves increasing endogenous insulin secretion and inhibiting common dietary enzymes

*Corresponding Author: Dr. A. Maruthupandian, Ethanopharmacological and Algal Biotechnology Laboratory, Department of Botany, School of Life Sciences, Periyar University, Salem-636011, Tamil Nadu. Email: pandianmdu82@gmail.com.

like α -amylase and α glucosidase. At the present study red seaweed *Portieria hornemannii* are evaluate the proximate composition of protein, carbohydrates, lipids, total, soluble, and insoluble fibre, moisture, ash, and physicochemical characteristics of the SWC, WHC, and OHC. Finally the seaweed *Portieria hornemannii* are used in anti diabetic activity of α -amylase enzyme inhibition assay.

MATERIALS AND METHODS

Sample collection

Portieria hornemannii, (Lyngbye) is a marine algae, the sample was collected in fresh and solidly during December 2017 and January 2018 of Kilakarai, Ramnad, Tamil Nadu, India (9°14'58" N/78°47'18" E). The collected sample was preserved in a polythene bag and transmitted to the lab inside that condition. After that, the marine algae was cleaned with fresh seawater and there in distilled water to remove all the unwanted impurities. Then the seaweed was immediately frozen and kept at a temperature of -20°C after being taken.

Proximate composition

All the proximate content was determine in standard method (AOAC, 2000). By using the oven method at 105°C, the moisture content was determined until a steady weight was attained. The (AOAC, 1995) was used to determine the crude protein concentration after converting

total nitrogen into crude protein using a conversion factor of 6.25. The carbohydrate content (%) was estimated by James, (1995). The seaweeds were incinerated for 16 hours at 550°C in a muffle furnace to estimate the amount of ash content using a gravimetric method. (Bligh and Dryer, 1959), a Soxhlet extractor was used to extract the lipid from seaweed powder using methanol (2:1, v/v).After drying the extract overnight in an oven at 80°C, the crude lipid content was determined gravimetrically. An enzymatic-gravimetric method was used to determine the amount of total, soluble, and insoluble dietary fiber in each sample (AOAC, 2000).

Physicochemical properties

Considering the experimental methodology used in a European collaborative research, the swelling and water holding capacity and oil holding capacity of seaweed samples were evaluated (Robertson *et al.*, 2000).

Swelling capacity (SWC)

The (SWC) Swelling capacity of algal sample was determined in (Kuniak and Marchessault, 1972) slightly modified. 10 ml measuring cylinder with 0.1 mL graduations and 0.02% sodium azide as a bacteriostatic was used to weigh the dry powdered sample (500 mg). After gently stirring to release any trapped air bubbles, it was left at room temperature for an additional 18 hours to allow the sample to settle. The sample's volume (ml) was determined, and SC was calculated as ml/g of dry material.



Figure 1. Morphology of *Portieria hornemannii*.

Water holding capacity (WHC)

The (WHC) water holding capacity of algae was determined in (Suzuki *et al.*, 1996) slightly modified. Five hundred mg of dry powdered materials were dissolved in 30 ml distilled water containing 0.02% sodium azide in a 50 ml centrifuge tube. After being stirred, the seaweed was kept at room temperature for 18 hours. WHC was estimated as g water/g of dry sample was centrifuge at 3,000× g for

20 min, after that supernatant was removed, and the pellet was weighed.

Oil holding capacity (ORC)

The (OHC) oil holding capacity of algae was evaluated by Caprez *et al.*, (1986) with a few minor changes. In each centrifuge tube, 3 g of seaweed samples were added together with 10.5 g of coconut oil. The tubes were stirred

for 30 minutes at room temperature (25°C). The mixture was then centrifuged at 2500 g for 30 minutes. The oil supernatant was then removed and measured. Then remove the oil supernatant was measured. The OHC of samples of seaweed was expressed as how much oil (1 g of sample) can be kept in 1 g (DW).

Preparation of algal extract *P. hornemannii*

The dried *P. hornemannii* was grounded and extracted for using Soxhlet apparatus for 5 hrs. After that 200 ml of methanol (1:10, weight to volume) and add 20 gm of algal sample were used in extracted from the seaweed, to ensuing crude extracts were filtered and then concentrated in a rotary evaporator. According to usage, the crude extracts were weighed and placed in a deep freezer (-20°C).

Anti diabetic activity : α - amylase enzyme Inhibition assay

α - amylase enzyme Inhibition assay was determined the method (Verspohl, 2002) .16 mM sodium acetate buffer 100 ml were stirred with 0.1 g of potato starch to produce a 0.1% w/v starch solution. To make the enzyme solution, 27 mg of α -amylase were dissolved in 100 ml of distilled water. The 3, 5-dinitro salicylic acid solution and sodium potassium tartrate solution were combined to produce the colorimetric reagent (96 mM). one ml of the starch solution was mixed with algal extracts at varying concentration (range from 25 to 400 g/mL) and left for 10 minutes, then add enzyme solution and allow reacting for 10 mins at 25°C in an alkaline environment. After adding 1 ml of colorimetric reagent to end the reaction, it was 5 minutes of incubation in a water bath, followed by cool to room temperature. The reaction mixture was diluted after being added to 10 mL of distilled water, and absorbance was measured at 540 nm. Control experiments, which used DMSO in place of extract and represented 100% enzyme activity, were carried out similarly. Similar tests were done using the standard drug.

Statistical analysis

The statistical test ANOVA was performed, where the data was expressed as mean $\pm$ standard deviation (SD) of triplicates of each assay. The statistical analysis was conducted using Origin Pro version 8.0.

RESULTS AND DISCUSSION

Protein, carbohydrates, lipids, total dietary fibre (TDF), soluble and insoluble fibre, moisture, and ash content of the *P. hornemannii* are presented in (Table 1). Total dietary fibre (TDF), soluble and insoluble fiber, moisture, and ash content were all estimated as part of the proximate composition (value stated in g/100g/DW). The *P. hornemannii* protein content (33.82 ± 1.48 g/100g/DW) is higher than other composition of carbohydrate, and lipid and TDF. Followed by the carbohydrate (22.09 ± 1.81 g/100g/DW), the lipid content is very low (3.62 ± 0.55 g/100g/DW). The total dietary fiber is very low compare with lipids (2.24 ± 0.39 g/100g/DW) then soluble and insoluble is also low (1.02 ± 0.05 , 1.22 ± 0.06 g/100g/DW). The ash and moisture content is (18.42 ± 1.03 , 12.96 ± 0.55 g/100g/DW) respectively. In previous literature (Wong and chaung, 2000) are reported in red seaweed *H. Japonica* protein 19.0 ± 0.36 , carbohydrate 4.28 ± 1.52 , lipid 1.42 ± 0.35 , TDF is 53.2 ± 0.56 the total dietary fiber (≥ 50 times higher in *P. hornemannii*) and ash and moisture is 22.1 ± 0.72 and 9.95 ± 0.27 g/100g/DW. Fleurence, 1999; Sanchez-machando *et al.*, (2004) are reported in the lower level of protein content is 5.46% DW and *H.elongata* it's also reported in lower level of protein (10.95% DW). The low protein content in (Mc.dermid and stuereke 2003) is reported in *Halymania recemosa* 21.2 % DW. Marinho Soriano *et al.*, (2010) was reported in *G. cervicornis* 23.0% DW. Danis *et al.*, (2010) are reported in very low level protein content of *Grateloupia turuturu* 22.9%DW. Ismail, (2017) are observed in *U. fasciata* protein content is (2.96%) and *C. officinalis* (1.37%), of the red seaweed lipid content maximum 5% below. (Gomez-Ordenez *et al.*, 2010) are reported in soluble, insoluble and total dietary fiber content of red seaweed *Mastocarpus* is higher (22.85%, 8.85% and 31.70% g/100g/DW) compare with *P. hornemannii*. Mohammed *et al.*, (2021) has also reported in total, soluble and insoluble fiber content is (31.49%, 23.61% and 7.88%) of red seaweed and this results also highest, compare with *P. hornemannii* (Gomez-Ordenez *et al.*, 2010) has reported in the ash content of *mastocarpus* (24.99). and Stevant *et al.*, (2017) are reported in ash content 25.6% then (Cofrades *et al.*, 2010) are reported in ash content for *nori* is (12 %). Mohammed *et al.*, (2021) has similar results ash content Dulce (25.63)

Table 1. Proximate composition of *Portieria hornemannii*.

Proximate composition	<i>Portieria hornemannii</i> g/100g
Protein	33.82 ± 1.48
Carbohydrate	22.09 ± 1.81
Lipid	3.62 ± 0.55
Total dietary fiber	2.24 ± 0.39
Soluble fiber	1.02 ± 0.05
Insoluble fiber	1.22 ± 0.06
Moisture	12.96 ± 0.55
Ash	18.42 ± 1.03

Table 2. Physicochemical properties of *P.hornemannii*.

Seaweed	SWC (ml occupied by sample/ dry sample)		WHC (g water hold/g, dry sample)		OHC (g oil retained/g sample)
	25°C	37°C	25°C	37°C	
<i>P.hornemannii</i>	13.02 ± 0.26	15.44 ± 0.18	7.35 ± 0.23	9.67 ± 0.32	3.23 ± 0.43

Table 3. α amylase enzyme activity.

Concentration $\mu\text{g/ml}$	Percentage inhibition of <i>P.hornemannii</i>
25	34.75 ± 1.25
50	40.73 ± 1.71
100	52.03 ± 1.27
200	66.25 ± 2.15
400	75.10 ± 1.95
IC50 value - 121.68	

and *nori* (17.24 %). Benjama and Masniyom, (2012) are shown in similar results of two red algae of *G.fisheri* and *G. tenuistipitata* and two different seasons of summer and rainy moisture content is (5.5% and 3.6% DW) compare with *P.hornemannii* its vary low respectively. The seaweeds are rich in dietary fiber (≥ 50 % DW). In previously study Darcy-vrillon, 1993) are reported in the seaweed powder have dietary fiber's physiological effects are correlated with physicochemical characteristics Wong, (2000). In seaweed are influenced in chemical structure of cell wall polysaccharides and their proteins are play in vital role of physicochemical properties of water holding capacity (WHC), oil holding capacity (OHC) and swelling capacity (SWC) in this study physicochemical capacity of *P.hornemannii* results are shown in Table 2 depicted in below. Physicochemical properties of *P.hornemannii* at 25°C of swelling capacity and water holding are ranged from (13.02 ± 0.26 ml/g DW and 7.35 ± 0.23 g/g DW) respectively. In previous report (Wong and Chaung, 2000) at 25°C of swelling capacity and water holding are range from (11.2% to 22.1 ml/g DW and 8.68-11.8% g/g DW) and *H.japonica* being highest SWC and WHC of (≤ 0.05 ANOVA). WHC of sample at 25°C (7.50 g/g DW) and *E. compressa* (9.50 g/g DW) Lahaye and Jegou, (1993) respectively. Additionally, the red seaweeds swelling capacity and water holding were equivalent to other commercial dietary fiber's SWC (9.90–24.0 ml/g DW) and WHC (6.60–9.00 g/g DW) Gonai and Martin-Carroan, (1998). In present study 37°C of SWC and WHC are ranged from (15.44 ± 0.18 ml/g DW, 9.67 ± 0.32 g/g DW) respectively, the results are shown in Table 2 depicted in below. In previous study seaweed samples WHC levels were equivalent to two seaweed *hijiki* and *kombu* (approximately 10.0 ± 12.0 g/g DW) (Suzuki *et al.*, 1996) obtained at the same temperature, although being lower than those of *wakame* (19.0 ± 44.0 g/g DW) (Suzuki *et al.*, 1996) and *L. digitata* (18.7 g/g DW) (Fleury & Lahaye, 1991) In closely related to *H.japonica* at 37°C of SWC and

WHC are ranged from (24.1 ml/g DW and 14.0 g/g DW) and *H.charoides* (20.7 and 12.4) g/g DW respectively.

Food formulation of used to oil holding capacity (OHC), a further functional food feature. Food products with a high oil capacity values allow the stabilization of food emulsions and foods with a lot of fat. In present study OHC are ranged from (3.23 ± 0.43) it's depicted in Table 2 respectively. Oil retention capacity was low for *Mastocarpus stellatus*, *Himanthalia elongata* (sea spaghetti), *Gigartina pistillata*, *Bifurcaria bifurcate* and *Laminaria saccharina* (sweet kombu), and all seaweed samples in previous reports (1.22-1.67 g/g dry weight of (Gomez-Ordóñez *et al.*, 2010). the seaweed of *P.hornemannii*, The physicochemical qualities of their fiber content are influenced by their anatomy and physiological characteristics Elleuch *et al.*, (2011). In present study *P. hornemannii* sufficient SWC, WHC, and OHC values indicated that they might be exploited as functional qualities in human diets or adjusted to change the texture and viscosity of food products.

The α -amylase enzyme activity are react simultaneously in human body to consume by intestine α - glucosidase absorbing glucose and breaking down starch via pancreatic α -amylase. In the present study the methanolic extract of *P.hornemannii* at the concentration 400 $\mu\text{g/ml}$ of α -amylase inhibition assay of (75.10 ± 1.95 $\mu\text{g/ml}$) and base concentration of 25 $\mu\text{g/ml}$ shown the result is (34.75 ± 1.25 $\mu\text{g/ml}$) respectively. These observances are shown in Table 3. The percent inhibitory activity increased in a dose dependent manner of increasing concentration against inhibited α -amylase enzyme activity IC₅₀ value 121.68 $\mu\text{g/ml}$ respectively. In previous report (Murugesan *et al.*, 2016) are shown the similar findings are made up 900 $\mu\text{g/ml}$ concentration of *P.hornemannii* and *S.fusiformis* the IC₅₀ value 430 $\mu\text{g/ml}$ and 175 $\mu\text{g/ml}$ respectively. The maximam observations of brown and red seaweed extracts, including several *Sargassum spp* and *Ishige okamurae*, have been reported in earlier studies

(Unnikrishnan *et al.*, 2015; Yang *et al.*, 2019), and various solvents of *Turbinaria decurrens*, *Padina pavonica* and *Sargassum muticum* (Ismail *et al.*, 2020). The red seaweed *P.hornemannii* has potential function of α -amylase enzyme activity.

CONCLUSION

In the current study can be concluded that the red seaweed *P.hornemannii* is a good quality of proximate composition of protein in high concentration. The physicochemical properties of SWC, WHC and OHC have highly useful dietary qualities, particularly for the treatment of diabetes. It is recommended that the active ingredient, which is responsible for the antidiabetic enzyme, from the *P. hornemannii* methanol extract in order to assess the in vivo study of anti diabetic rats.

ACKNOWLEDGEMENT

The authors would like grateful to the Department of Botany, Periyar University Salem-11 for the necessary facilities to carry out this research. This study was supported by Periyar University under the grant of a university research fellowship (PU/AD-3/URF/2016).

REFERENCES

- Mendes, M. C., Navalho, S., Ferreira, A., Paulino, C., Figueiredo, D., Silva, D., Gao, F., Gama, F., Bombo, G., Jacinto, R. and Aveiro, S.S., & Speranza, L. G. (2022). Algae as food in Europe: an overview of species diversity and their application. *Foods*, 11(13), 1871.
- Unnikrishnan P S, Suthindhiran K and Jayasri M A 2015 Antidiabetic potential of marine algae by inhibiting key metabolic enzymes. *Journal Frontiers in Life Science*, 8, 148-159.
- Nickavar B and Yousefian N (2011) Evaluation of α -amylase inhibitory activities of selected antidiabetic medicinal plants. *Journal of Vertebrates Lebensm*, 6, 191-195.
- Wong, K. H., & Cheung, P. C. (2000). Nutritional evaluation of some subtropical red and green seaweeds: Part I—proximate composition, amino acid profiles and some physico-chemical properties. *Food chemistry*, 71(4), 475-482.
- Fleurence, J. (1999). Seaweed proteins: Biochemical, nutritional aspects and potential uses. *Trends in Food Science and Technology*, 10(1), 25-28.
- Sanchez-Machado, D. I., López-Cervantes, J., López-Hernández, J., & Paseiro-Losada, P. (2004). Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food Chemistry*, 85(3), 439-444.
- Gómez-Ordóñez, E., Jiménez-Escrig, A., & Rupérez, P. (2010). Dietary fibre and physicochemical properties of several edible seaweeds from the northwestern Spanish coast. *Food Research International*, 43(9), 2289-2294.
- Ismail, A. R. (2017). The influence of perceived social media marketing activities on brand loyalty: The mediation effect of brand and value consciousness. *Asia pacific Journal of Marketing and Logistics*, 29(1), 129-144. <http://dx.doi.org/10.1108/APJML-10-2015-0154>.
- Denis, C., Morañais, M., Li, M., Deniaud, E., Gaudin, P., Wielgosz-Collin, G., Barnathan, G., Jaouen, P. and Fleurence, J. (2010). Study of the chemical composition of edible red macroalgae Grateloupia turuturu from Brittany (France). *Food Chemistry*. 119, 913-917.
- McDermid, K.J. and Stuercke, B. (2003). Nutritional composition of edible Hawaiian seaweeds. *Journal of Applied Phycology*. 15, 513-524.
- Marinho-Soriano, E., Fonseca, P.C., Carneiro, M.A.A. and Moreira, W.S.C. (2006). Seasonal variation in the chemical composition of two tropical seaweeds. *Bioresource Technology*. 97, 2402-2406.
- Mohammed, H. O., O'Grady, M. N., O'Sullivan, M. G., Hamill, R. M., Kilcawley, K. N., & Kerry, J. P. (2021). An Assessment of Selected Nutritional, Bioactive, Thermal and Technological Properties of Brown and Red Irish Seaweed Species. *Foods*, 10(11), 2784.
- Stévant, P.; Marfaing, H.; Rustad, T.; Sandbakken, I.; Fleurence, J.; Chapman, A (2017). Nutritional value of the kelps *Alaria esculenta* and *Saccharina latissima* and effects of short-term storage on biomass quality. *Environmental Biology and Fisheries*, 29, 2417–2426.
- Cofrades, S, López, I.L, Bravo, L, Ruiz-Capillas, C, Bastida, S.; Larrea, M.T, Jimenez-Colmenero, F (2010). Nutritional and Antioxidant Properties of Different Brown and Red Spanish Edible Seaweeds. *Food Science and Technology International*, 16, 361-370.
- Benjama, O., & Masniyom, P. (2012). Biochemical composition and physicochemical properties of two red seaweeds (*Gracilaria fisheri* and *G. tenuistipitata*) from the Pattani Bay in Southern Thailand. *Sonklanakarin Journal of Science and Technology*, 34(2), 223.
- Lahaye, M., & Jegou, D. (1993). Chemical and physico-chemical characteristics of dietary fibers from *Ulva lactuca* (L.) Thuret and *Enteromorpha compressa* (L.) Grev. *Journal of Applied Phycology*, 5, 195-200.
- Gonai, I., & Martin-Carroan, N. (1998). In vitro fermentation and hydration properties of commercial dietary fiber-rich supplements. *Nutrition Research*, 18, 1077-1089.
- Suzuki, T., Ohsugi, Y., Yoshie, Y., Shirai, T., & Hirano, T. (1996). Dietary fiber content, water holding capacity

- and binding capacity of seaweeds. *Fisheries Science*, 62, 454-461.
- Fleury, N., & Lahaye, M. (1991). Chemical and physico-chemical characterization of fibers from *Laminaria digitata* (Kombu Breton): a physiological approach. *Journal of Science and Food Agriculture*, 55, 389-400.
- Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C. and Attia, H. 2011. Dietary fibre and fibre-rich byproducts of food processing: Characterisation, technological functionality and commercial applications: A review. *Food Chemistry*. 124, 411–421.
- Unnikrishnan, P. S., Suthindhiran, K., & Jayasri, M. A. (2015). Antidiabetic potential of marine algae by inhibiting key metabolic enzymes. *Frontiers in Life Science*, 8(2), 148-159.
- Yang, H.-W., Fernando, K. H. N., Oh, J.Y., Li, X., Jeon, Y.J., & Ryu, B. (2019). Anti-obesity and anti-diabetic effects of *Ishige okamurae*. *Marine Drugs*, 17(4), 202.
- Murugesan, S., Bhuvaneswari, S., & Sivamurugan, V. (2016). Evaluation of in vitro antidiabetic activity of red seaweed *Portieria hornemannii* (Lyngbye)(Silva) and *Spyridia fusiformis* (Wulfen). *World Journal of Pharmaceutical Sciences*, 4(6), 415-419.
- Sanger, G., Rarung, L. K., Damongilala, L. J., Kaseger, B. E., & Montolalu, L. A. D. Y. (2019). Phytochemical constituents and antidiabetic activity of edible marine red seaweed (*Halymenia durvillae*). In IOP conference series: earth and environmental science Vol. 278(1) pp. 012069.
- Basheer, S., Huo, S., Zhu, F., Qian, J., Xu, L., Cui, F., & Zou, B. (2020). Microalgae in human health and medicine. In *Microalgae biotechnology for food, health and high value products*, 149-174.
- Caprez, A., Arrigoni, E. V. A., Amadò, R., & Neukom, H. (1986). Influence of different types of thermal treatment on the chemical composition and physical properties of wheat bran. *Journal of Cereal Science*, 4(3), 233-239.
- Kuniak, L., & Marchessault, R. H. (1972). Study of crossing-linking reaction between epichlorhydrin and starch. *Starke*, 4, 110-116.
- Suzuki, T., Ohsugi, Y., Yoshie, Y., Shirai, T., & Hirano, T. (1996). Dietary fiber content, water holding capacity and binding capacity of seaweeds. *Fisheries Science*, 62, 454- 461.
- James, (1995). C.S. James Analytical chemistry of foods. Chapman and Hall, New York.
- AOAC, (2000). Method 992.23, In “Official Methods of Analysis”, 17th Ed., AOAC International, Gaithersburg, MD.
- AOAC (1995). Official methods of analysis (16th ed.). Washington, DC, USA; Association of Official Analytical Chemists.
- Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian journal of Biochemistry and Physiology*, 37(8), 911-917.
- Robertson, J.A., de Monredon, F.D., Dysseler, P., Guillon, F., Amado, R. & Thibault, J.F. 2000. Hydration properties of dietary fibre and resistant starch: A European collaborative study. *LWT - Food Science and Technology*, 33(2), 72-79.
- Verspohl EJ, 2002. Recommended testing in diabetes research. *Planta Medicine*, 68, 581-590.
- Khalid, W., Arshad, M. S., Jabeen, A., Muhammad Anjum, F., Qaisrani, T. B., & Suleria, H. A. R. (2022). Fiber-enriched botanicals: A therapeutic tool against certain metabolic ailments. *Food Science & Nutrition*.1-16.
- El-Beltagi, H. S., Mohamed, A. A., Mohamed, H. I., Ramadan, K., Barqawi, A. A., & Mansour, A. T. (2022). Phytochemical and Potential Properties of Seaweeds and Their Recent Applications: A Review. *Marine Drugs*, 20(6), 342.
- Darcy-Vrillon, B. (1993). Nutritional aspects of the developing use of marine macroalgae for the human food industry. *International Journal of Food Sciences and Nutrition*, 44, S23-S35.