ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 2, pp: (263-268), Month: April - June 2018, Available at: www.researchpublish.com

Vitamin content of marine red alga *Botryocladia leptopoda* (J.Agardh) Kylin

Potential use as a source of vitamins in nutrition

Gajalakshmi, D[1], Shettu, N[1] and Murugesan, S[2*]

[1]PG and Research Department of Zoology, Pachaiyappa's College, Chennai - 600 030.

[2]Division of Algal Biotechnology and Bionano Technology, PG and Research Dept of Botany, Pachaiyappa's College, Chennai - 600 030.

[*]*Email: smurugesan5@gmail.com.*

Abstract: **Seaweed, being a rich source of basically different bioactive compounds with significant nutraceutical properties, can be used as an ingredient to supplement nourishes with functional compounds. The vitamin examination proposes that the marine red alga *Botryocladia leptopoda* is rich in vitamin C and vitamin B complex. Consequences of the investigation proposing that the *B. leptopoda* have high nutritional value and hence it could be used as a nutritional supplement.**

Keywords: **Vitamin, Marine alga, *Botryocladia leptopoda*, Nutraceutical.**

1. INTRODUCTION

Algae has existed on earth since its start, however, its prospective advantages have as of late been comprehended and consequently, it is presently being considered to play a main position in solving some of the most serious troubles of the world. Seaweeds are one of the vital marine living resources in the world. However, the expense of algae production is an obstruction in production of algae a normal candidate for the expansion of a range of products (Singh and Shaishav Sharma, 2017).

Macroalgae have been a source of food, feed and medicine in the east as well as in the west, since old circumstances (Chapman and Chapman, 1980). Seaweed, being a rich source of basically assorted bioactive compounds with significant nutraceutical properties, can be used as an ingredient to supplement food with functional compounds. Around 150 species of seaweeds are used as nourishment worldwide and more than 100 species for seaweed gum production. Marine macroalgae are viewed today to be an essential for a variety of products: they are useful in aquaculture (Strohecker *et al.*,1963), in the bioconversion of solar energy (Goldman, 1979), as a source of glycerol, carotene and dry algal meal (Ben-Amotz and Avron, 1980.), pharmaceutical products (Aaronson *et al.*, 1980), a raw material for Single Cell Protein (SCP) (Fabregas and Herrero, 1985), or as provide of minerals in fish diets (Fabregas and Herrero, 1986).

Today, seaweed utilization has expanded because of their natural composition. They were recorded to have numerous helpful nutritive bioactive compounds such as vitamins (ascorbic and β-carotene), polyphenols, pigments, minerals, fibers and polysaccharides (Lahaye, 1991). They are low in fat and in calorific value with high levels of essential fatty acids and essential amino acids notwithstanding around 80-90% water. Marine macroalgae would thus be considered and are able to characterize a non-conventional source of vitamins or a vitamin supplement for animal or human nourishment.

Seaweeds are a good source of water soluble (B1 , B2 , B12, C) β-carotene with vitamin A, D and fat-soluble (E) vitamins. To ensure that the adequate intake of all vitamins is received in the diet, people can consume foods enriched with vitamins, for example, in the form of functional foods with vitamins as nutraceuticals, extracted from natural sources such as seaweeds. Seaweed vitamins are important not only due to their biochemical functions and antioxidant activity, but also due to other health advantages, such as diminishing of blood pressure (vitamin C), diminishing the danger of cardiovascular diseases of cancer (vitamins E and C, carotenoids) (Skrovankova, 2011; Swarnalatha, 2018).

As of late a considerable measure of research has been done on the improvement of new health foods from algal biomass. Algal biomass and extracts are used widely in the formulations of gels, capsules, tablets, gums, bars, snacks, pastas, drinks, and beverages (Goh *et al.*, 2009). Algal biomass production can be enhanced through embracing modern methods of enhancing biomass coupled to technological interventions for functional food applications (Ranga Rao and Ravishankar, 2018). The major objective of the present investigation was to assess the nutritional value of marine red alga *Botryocladia leptopoda* (J.Agardh) Kylin by examinning their vitamin content.

2. MATERIALS AND METHODS

2.1 Collection and Authentication

The red seaweed, *Botryocladia leptopoda* (J.Agardh) Kylin was gathered from Mandapam, Gulf of Mannar, Mandapam Coast, Ramnad, South India,Tamil Nadu and validated by Dr.R.Veeragurunathan, Scientist, CSIR-Central Salt & Marine Chemicals and Research Institute, Mandapam, Tamilnadu, India.

2.1.1 Processing of collected sample

The seaweeds collected were cleaned with seawater to eliminate dirt and debris beside with epiphytes, sand particles, and shells. Then, the seaweeds were washed with running tap water followed by distilled water and dried totally at room temperature. The subsequent dried material was roughly powdered (passing through 40 size sieve) and utilized for additional contemplates.

2.1.2 Preparation of extract

The red seaweed *B. leptopoda* extract was set up by adding methanol to round bottom flask followed by the inclusion of 50 g of seaweed powder and was subjecting to soxhlet apparatus at 64°C for 72 hrs. The extract (representing both lower polar, polar and non-polar components) of *B. leptopoda* were pooled together and vanished under condensed pressure using a rotary flash evaporator (Superfit, India). The crude extract was quantified and used for further examination.

2.2 Estimation of vitamin B complex

The vitamin B group was extracted according to a previous standard method (AOAC, 1990). In concise, seaweed powder (2 g) was positioned in 25 mL of H_2SO_4 (0.1 N) solution and incubated for 30 min at 121°C. Then, the contents were cooled and changed in accordance with pH 4.5 with 2.5 M sodium acetate and 50 mg Taka diastase enzyme was included. The preparation was stored at 35°C overnight. The blend was then filtered through a Whatman No.4 filter and the filtrate was diluted with 50 mL of clean water and filtered again through a micropore filter (0.45 µm). Twenty microliters of the filtrate was infused into the HPLC system. Evaluation of vitamin B content was able by contrast to vitamin B standards. Standard stock solutions, thiamine, riboflavin, niacin, pyridoxine, and cobalamin were prepared as reported previously (Aslam *et al.*, 2008; Ringling and Rychlik, 2013). Chromatographic separation was accomplished on a reversed phase-(RP-) HPLC column (Agilent ZORBAX Eclipse Plus C18; 250 × 4.6 mm i.d., 5 µm) through the isocratic release mobile phase (A/B 33/67; A: MeOH, B: 0.023 M H_3PO_4, pH = 3.54) at a run rate of 0.5 mL/min. Ultraviolet (UV) absorbance was recorded at 270 nm at room temperature (Marzougui *et al.*,2009).

2.2.1 Estimation of vitamin C

Vitamin C was separated by an adjustment of a distributed strategy method (Babarinde and Fabunmi, 2009). The seaweed powder (10 g) was blended and homogenized with an extracting solution containing metaphosphoric acid (0.3 M) and acetic acid (1.4 M). The blend was kept in a conical flask and agitated at 10,000 rpm for 15 min. The blend was then filtered through a Whatman No.4 filter and samples were extracted in triplicate. The ascorbic acid standard was ready by dissolving 100 mg of L-ascorbic acid in a metaphosphoric acid (0.3 M)/acetic acid (1.4 M) solution at a end concentration of 0.1 mg/mL. The calibration line was changed over to a straight range based on four estimated concentration levels.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 6, Issue 2, pp: (263-268), Month: April - June 2018, Available at: www.researchpublish.com

Measurement of the ascorbic acid content was performed on an Agilent HPLC system. Chromatographic separation was accomplished on an RP-HPLC column through isocratic delivery of a mobile phase (A/B 33/67; A: 0.1 M potassium acetate, pH = 4.9, B: acetonitrile: water [50 : 50]) at a flow rate of 1 mL/min. UV absorbance was recorded at 254 nm at room temperature.

2.3 Statistical analysis

All the experiments were carried out in triplicates and the data of vitamin investigation subjected to analysis of variance (ANOVA) using SPSS version (17.0). The outcomes were expressed as mean ± standard error (SE).

3. RESULTS AND DISCUSSION

Vitamins are necessary in the human body for different chemical and physiological functions. Seaweeds are usually a good supply of B complex (B1, B2, B3, B6, B9 and B12) (Kim and Taylor, 2011). Vitamins are vital for all organisms as they offer the precursors to enzyme cofactors which are significant for metabolism. Seaweeds contain both water-soluble (Vitamin-B and Vitamin-C) and fat-soluble vitamins (Vitamins-A, D, E and K) (Sanchez-Machado *et al*., 2004) Vitamin content of experimental algae was shown in Table. 1.

Table.1 Vitamin content of *Botryocladia leptopoda.*

S.No	**Name of Vitamins**	***µg/g dry wt***
1	Vitamin B1	2.34 ± 0.02
2	Vitamin B2	1.18 ± 0.17
3	Vitamin B3	12.40 ± 0.17
4	Vitamin B6	0.33 ± 0.12
5	Vitamin B9	1.55 ± 0.01
6	Vitamin C	5.45 ± 0.18
P-Value		0.000
F-Value		1541.00

Vitamin B contains a variety of vitamin B complex, which is essential for cell metabolism, red blood cell development and different other biological functions. Seaweeds are usually a excellent source of B group vitamins (B1, B2, and B12) (Kim and Taylor, 2011).

Vitamin B1 (Thiamine) in view of its high oxidative metabolism, thiamine deficiency, especially focuses on the nervous system and the heart. Thiamine acting as a central position in the production of energy from carbohydrates. In the present investigation, the vitamin B1 content of the *B. leptopoda* was observed to be 2.34 ± 0.02 µg/g dry wt. It was higher than those revealed by Sánchez-Machado *et al.*, (2004) with regarding to the marine algae *Palmaria sp.*, *Porphyra* sp. Thiamin (B1) co-enzymes works on the metabolism of carbohydrates and cleft chain amino acids. Thiamin insufficiency causes beri-beri, polyneuritis.

Vitamin B2 (Riboflavin) is a water soluble member of the vitamin B complex. Riboflavin is a precursor of the coenzymes riboflavin mononucleotide and flavin adenine dinucleotide, which are additionally associated with energy metabolism (Mahan and Escott-Stump, 2000). In the present investigation, the vitamin B2 content of the *B. leptopoda* was observed to be 1.18 ± 0.17 µg/g dry wt. The higher content of vitamin B2 found in red alga *Porphyra sp.*, (Kim and Taylor, 2011). Vitamin B2 is suggested for treatment of the effect of aging, chronic fatigue syndrome and anemia (Fayaz *et al.*, 2005).

The vitamin B2 content in the present investigation was higher than the previous reports of marine red algae *Spyridia fusiformes* (Bhuvaneswari *et al.*, 2013), *T. glomerulata* (Mohanapriya *et al.*, 2017). Vitamin B2 is observed to be a higher concentration in red algae than in brown and green algae (Ragan and 1986). Vitamin B2 lack may cause severe health disorders such as megaloblastic anemia and neuropsychiatric disorders (Misurcova, 2011).

Vitamin B3 is known as niacin are necessary for biological processes such as mental alertness. Niacin is a co-enzyme or co-substrate in several biological reduction and oxidation reactions thus necessary for energy metabolism. It is helpful in

the treatment of pellagra with diarrhea, dermatitis and dementia. It also acts as a stress reducer and brings sown the cholesterol level and helps in turning around the atherosclerosis. The vitamin B3 content of *B. leptopoda* was observed to be 12.40 ± 0.17 µg/g dry wt and it was a less amount of contrasted to that of the vitamin B3 content of *T. glomerulata* (Mohanapriya *et al.*, 2017). The consequence of the present examination matched with the past report of Bhuvaneswari *et al.*, (2013) in *Chondrococcus hornemannii* and *Spyridia fusiformis*.

Vitamin B6 (Pyridoxine) is typically stored in the body as pyridoxal-5'-phosphate (PLP), which is the co-enzyme form of vitamin B6. The vitamin B6 content of *B. leptopoda* was seen as 0.33 ± 0.12 µg/g dry wt. The present examination was matched with the previous report in the red algae *T. glomerulata* (Mohanapriya *et al.*, 2017); *Champia parvula* (Vinoth kumar *et al.*, 2015); *Laurencia papillosa* (Sathish Kumar and Murugesan, 2018). Vitamin-B_6 is associated with breaking down carbohydrates and transforming it into vitality and keeps a healthy brain working. Vitamin B_6 is synthesized in algae by the activity of Glutamate-1-semialdehyde 2, 1-aminomutase (Cheltsov *et al.*, 2003).

The investigated alga *B. leptopoda* contains 1.55 ± 0.01 µg/g dry wt of Vitamin B9 (Folic acid or Folate or Phenyl L-glutamic acid). Vitamin B9 is broadly found in algae in higher concentrations in green and red algae than in brown algae (Ragan and 1986). The maximum content of vitamin B9 in seaweed estimated to date is seen in red alga *Porphyra* sp. (134 µg/100 g dw). It has additionally been accounted that a high content of this vitamin is seen in green alga *Enteromorpha* sp., (Kim and Taylor, 2011). Vitamin B9 is suggested for treatment of the effects of aging, chronic fatigue syndrome and anemia (Fayza *et al.*, 2005). Vitamin B9 also helps in erythropoiesis, the generation of red blood cells (National Academy of Sciences, 1998). The vitamin B basically necessary for the improvement of human body is found in enormous plenitude in all phaeophyceae.

Vitamin-C is copious in *B. leptopodaa* (5.45 ± 0.18 µg/g dry wt.). It is available in vast amounts in the red alga *Gracilaria sp.*, and adds to the confirmation that the red seaweeds add to nutritional intake in a balanced diet (Institut de Phytonutrition, 2004). The vitamin C assumes an essential part as antioxidant and intensification the immune system and the nearness of vitamin C also reported in the marine red algae *Gelidiella acerosa, Porphyra umbilicalis, Palmaria palmata* (Haidara *et al.*, 2006; Anantharaman *et al.*, 2011). A red alga (*Porphyra*) was generally used to prevent scurvy, caused by vitamin C inadequacy (Karleskint *et al.*, 2012).

Moreover, vitamin-C, has other medical advantages such as radical scavenging, strengthening of the immune system, and antiaging activity (Witting and Stocker 2003; Haidara *et al.*, 2006). Vitamin C for protection to infections. Vitamins have various biochemical functions. Vitamins have hormone-like functions as regulators of mineral metabolism or regulators of cell and tissue development and separation. The vitamin C content of nori is around 1.5 times that of oranges and 75% of the protein and carbohydrates are digestible by humans, which is very high for seaweeds.

B-complex and C are water soluble vitamins occurs in plants, which constantly require in our diets as they are not stored in the body and are discharged in the urine. Ortiz *et al* (2006) revealed that 100 g seaweed gives more than the daily necessity of vitamins A, B2, B12, and two-thirds of the vitamin C requirement. Moreover, it also obviously offsets the inhibition of iron absorption by phytates in the diet.

Algal foods are rich in vitamins. Numerous sea vegetables like *Porphyra umbilicalis*, *Himanthalia elongata*, and *Gracilaria changii* contain levels of vitamin C similar to normal vegetables such as tomatoes and lettuce (Ferraces-Casais *et al.*, 2012), while the vitamin C content depicted for the brown seaweed *Eisenia arborea* approaches those described for mandarin oranges (Hernandez-Carmona *et al.*, 2009).

4. CONCLUSION

Vitamins are viewed as imperative supplements in foods and carry out specific functions fundamental for wellbeing however their every day necessities are minute. The vitamin composition differs with numerous factors, particularly species, geographic area, season and environmental parameters. The outcome of the examination requirements that *B. leptopoda* has more prominent nutritional value and could be used as an incredible nutritional supplement. Seaweeds can give a dietary option because of its nutritional and commercial value which, enhanced by improving the quality and extending the scope of seaweed based products. Further investigation is necessary in the utilization and sensory perceptions of these seaweeds.

REFERENCES

[1] Singh, R. N and S. Sharma. 2017. Utilization of Fish Excreta for Algae Production. Journal of Alternate Energy Sources and Technologies. 8(3): 9–17.

[2] Chapman, V. J. and Chapman, D. J., 1980. Seaweeds and Their Uses. (3rd ed). Chapman and Hall, New York. 334 p.

[3] Strohecker, R. and H.M. Henning. 1963. Vitamin Bestimmungen. E. Merck, Darmstadt, Verlag Chemie GmbH, Weinheim/Bergstrasse.

[4] Goldman, J.C. 1979. Outdoor algal mass culture. I. Applications. Water Res. 13: 1–9.

[5] Ben-Amotz, A and Avron, M. 1980. Glycerol, β-carotene and dry algal production by commerical cultivation of *Dunaliella*. In: Algae Biomass. Production and Use (Shelef G. and C.J. Soeder, eds.), pp. 603–610, Elsevier/North Holland Biomedical Press, Amsterdam.

[6] Aaronson S., Berner, T and Dubinsky, Z. 1980. Microalgae as source of chemicals and natural products. In: Algae Biomass. Production and Use (Shelef, G. and C.J. Soeder, eds.), pp. 575–602, Elsevier/North Holland Biomedical Press, Amsterdam.

[7] Fabregas, J and Herrero, C. 1985. Marine microalgae as a potential source of single cell protein (SCP). Appl. Microbiol. Biotechnol 23: 110–113.

[8] Fabregas, J. and Herrero, C. 1986. Marine microalgae as a potential source of minerals in fish diets. Aquaculture 51: 237–243.

[9] Lahaye. M. 1991. "Marine Algae as Sources of Fibers: Determination of Soluble and Insoluble Dietary Fiber Contents in Some Sea Vegetables", J. of the Sci of Food and Agri. 54(4): 587-594.

[10] Skrovankova, S. 2011. Seaweed Vitamins as Nutraceuticals. *Adv Food Nutr Res*. 64: 357-369.

[11] Swarnalatha, G. 2018. Comparison of nutritive value of seaweed with other terrestrial foods—a review. *IJFANS*. 7(1): 61-70.

[12] Goh, LP, Loh, SP, Fatimah, MY and Perumal, K. 2009. Bioaccessibility of carotenoids and tocopherols in marine microalgae, Nannochloropsis sp and Chaetoceros sp. Malaysian Journal of Nutrition 15(1): 77-86.

[13] Ranga Rao, A and Ravishankar, G.A. 2018. Algae as Source of Functional Ingredients for Health Benefits. Agri Res & Tech: Open Access J 14(2): 1-4.

[14] AOAC. International, Official Methods of Analysis, Arlington, Va, USA, 15th edition, 1990.

[15] Aslam, J, Mohajir, M. S. Khan, S. A. and Khan, A. Q. 2008. "HPLC analysis of water-soluble vitamins (B1, B2, B3, B5, B6) in in vitro and ex vitro germinated chickpea (Cicer arietinum L.)," African Journal of Biotechnology, vol. 7, no. 14, pp. 2310–2314.

[16] Ringling, C and Rychlik, M. 2013. "Analysis of seven folates in food by LC-MS/MS to improve accuracy of total folate data," European Food Research and Technology, vol. 236, pp. 17–28.

[17] Marzougui, N. Guasmi, F. Mkaddem, M. et al., 2009. "Assessment of Tunisian Trigonella foenum graecum diversity using seed vitamin B6, B1, B9 and C contents," Journal of Food, Agriculture and Environment, vol. 7, no. 1, pp. 56–61.

[18] Babarinde, G. O. and Fabunmi, O. A. 2009. "Effects of packing materials and storage temperature on quality of fresh okra (*Abelmoschus esculentus L.*) fruit," Agricultura Tropica et Subtropica, vol. 42, pp. 151–156.

[19] Kim, S. K and Taylor, S. 2011. Marine medicinal foods: implications and applications, macro and microalgae. In: Kim SK, editor. *Food and nutrition research.* San Diego, CA: Academic Press. 358–363.

[20] Sanchez-Machado, D. I., Lopez-Hernandez, J., Paseiro- Losadam, P and Lopez-Cervantes, J. 2004. An HPLC method for the quantification of sterols in edible seaweeds. *Biomed. Chromatogr*. 18: 183-190.

[21] Mahan. K. L and Escott-Stump. S. 2000. *Nutrición y dietoterapia, de Krause*. Madrid, Spain: McGraw-Hill Interamericana.

[22] Fayaz, K., Namitha, K. N., Chidambara, M. M., Mahadeva, S. R., Sarada, S. K and Ravishankar, S. 2005. Chemical composition Iron bioavailability and antioxidant activity of *Kappaphycus alvarezii* (Doty). *J. Agric. Food Chem.* 53: 792-797.

[23] Bhuvaneswari, S and Murugesan, S. 2013. Biochemical composition of seaweeds along southeast coast of Tamil Nadu, India. *International Journal of Biology and Pharmacy and Allied Sciences.* 2(7): 1430-1436.

[24] Mohanapriya, P and Murugesan S. 2017. Biochemical composition of *Tolypiocladia glomerulata* (C. Agardh) F. Schmitz. *WJPLS*. (3):59-162.

[25] National Academy of Sciences. Institute of Medicine. Food and Nutrition Board, ed. 1998. "Chapter 8 - Folate". Dietary Reference Intakes for Thiamine, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline. Washington, D.C.: National Academy Press.

[26] Ragan, M. A and Glombitza, K.W. 1986. Phlorotannins, brown algal polyphenols. Progress in Phycological Research. 4: 129-147.

[27] Misurcova, L. 2011. Chemical composition of seaweeds. In: Kim SK, editor. Handbook of marine macroalgae: *Biotechnology and applied phycology*. West Sussex, UK: John Wiley & Sons. pp.608.

[28] Vinoth Kumar, R, Murugesan, S and Bhuvaneswari, S. 2015. Phytochemical analysis of red alga *Champia parvula* (C. Agardh) collected from Mandapam coast of Tamil Nadu, India. *International Journal of Advances in Pharmaceutics*. 4 (3): 15-20.

[29] Sathish Kumar, B and Murugesan, S. 2018. Evaluation of fatty acid, mineral and vitamin compositions of *Laurencia papillosa* from the South East coast of India. *IAJPS*. 05 (04): 2681-2687.

[30] Cheltsov, A. V. Guida, W. C and Ferreira, G. C. J. Biol. Chem. 278 (2003) 27945– 27955.

[31] National Academy of Sciences. Institute of Medicine. Food and Nutrition Board, ed. 1998. "Chapter 8 - Folate". Dietary Reference Intakes for Thiamine, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline. Washington, D.C.: National Academy Press.

[32] Institute de Phytonutrition. 2004. Functional, health and therapeutic effects of algae and seaweed. Institute Phytonutrition electronic database. Version 1.5. Beausoleil, France.

[33] Haidara MA, Yassin HZ, Rateb MA, Ibrahim IM, El-Zorkan MM, Roshd NK. 2006. The potential protective effects of vitamin C on glucose homeostasis and muscle function in STZ-induced diabetic rats. In: Peel T, editor. *Vitamin C: new research*. New York: Nova Publishers. p 41–57.

[34] Anantharaman, P., Devi, G. K., Manivannan, K. & Balasubramanian, T. 2011 Vitamin-C content of some marine macroalgae from Gulf of Mannar Marine Biosphere Researve, south east coast of India. Plant Archives 11(1): 343-346.

[35] Karleskint, G., Turner, R and Small, J. 2012. Multicellular primary producers. In: Ryder M, editor. Introduction to marine biology. Belmont, M. A. *Cengage Learning*. P. 157–189.

[36] Witting PK, Stocker R. 2003. Ascorbic acid in artherosclerosis. In: Asard H, May J, Smirnoff N, editors. *Vitamin C: Its functions and biochemistry in animals and plants*. Oxfordshire, UK: Garland Science/BIOS Scientific Publishers. p 261–91.

[37] Ortiz J, Romero N, Robert P, Araya J, Lopez-Hernández J, Bozzo C, Navarrete E, Osorio A, Rios A. Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. Food Chem. 2006;99:98–104.

[38] Ferraces-Casais P, Lage-Yusty MA, de Quiros ARB, Lopez-Hernandez, J. 2012. Evaluation of bioactive compounds in fresh edible seaweeds. Food Anal Methods 5:828–834.

[39] Hernandez-Carmona, G, Carrillo-Dominguez, S, Arvizu-Higuera, D.L., Rodriguez-Montesinos, Y.E., Murillo-Alvarez, J.I., Munoz-Ochoa, M., Castillo-Dominguez, R.M. 2009. Monthly variation in the chemical composition of Eisenia arborea JE Areschoug. J Appl Phycol 21: 607–616.