

## Preliminary evaluation of Nigeria coastal line seaweeds for the alginate content and biochemical constituents

Omodele Ibraheem,<sup>1,\*</sup> Toluwase O. Komolafe,<sup>1</sup> Elisha Bawa,<sup>2</sup> James O. Oluwole<sup>2</sup>

<sup>1</sup> Department of Biochemistry, Federal University Oye-Ekiti, PMB 373, Oye-Ekiti, Ekiti State, Nigeria

<sup>2</sup> Nigeria Navy Defence Head Quarter Complex, Plot 1092, Mohammadu Buhari Way, Area 7, Garki, FCT, Abuja, Nigeria

ORIGINAL RESEARCH ARTICLE

### ABSTRACT

Among the numerous polysaccharides embedded in seaweeds cell wall is alginate and it's found abundantly in brown seaweeds spp. Although Nigeria coastal line is enormously blessed with seaweeds, it hasn't been earnest for exploitation of alginate; an expensive polysaccharide used in many industrial applications. This research work therefore seeks to establish a framework towards the extraction of alginate from these seaweeds and evaluation of the biochemical compositions. Using established experimental protocols, results showed that Nigeria coastal shoreline seaweeds that yielded 13.34% is similar to other seaweeds exploited for alginate content in other countries shorelines. Furthermore, biochemical components such as carbohydrate, protein, lipid and total ash contents that gave 19%, 1.44%, 2.83% and 26%, respectively were within the ranges as observed in other seaweeds with the exception of protein content that gave a low yield. This study thus points out the exploitable potential of Nigeria coastal shoreline seaweeds and therefore cognate efforts should be geared towards mass production of alginate for various industrial applications, thus translating this supposed natural nuisance to wealth. Owe to the rich biochemical composition, these seaweeds may be used to boost quality of animal feeds, perhaps in future for human consumption; after very thorough toxicological screenings have been carried out. The seaweeds debris may further be employed as organic fertilizer and also as source of carbon for second generation bioethanol production.

### KEYWORDS

alginate; hydrocolloid; macroalgae; seaweeds; shoreline

## 1. INTRODUCTION

The marine environment consists of diverse plant-like organisms, generally among them there are macroalgae which are also known as seaweeds. They are found attached to rock or other hard substrata in coastal areas (Kılınc et al., 2013). Within the marine environment, diversities of these seaweed occur and are classified into divisions based on various properties such as their pigmentation, chemical nature of photosynthetic storage products, their organization of photosynthetic membranes, and other morphological features (Madhu, 2015). Traditionally, they belong to three basic groups (Figure 1), which are namely brown algae (phylum: Ochrophyta, classes: Phaeophyceae, about 1750

species), green algae (phylum: Chlorophyta, classes: Bryopsidophyceae, Chlorophyceae, Dasycladophyceae, Prasinophyceae, and Ulvophyceae; about 1200 species) and red algae (phylum: Rhodophyta, about 6000 species) (King, 2007). All seaweeds at some stage in their life cycles are unicellular, as spores or zygotes, and may be temporarily planktonic (Kılınc et al., 2013). Generally, seaweeds are known to flourish and survive more in temperate waters. Various macroalgal genera are postulated to have originated from the Indo-Pacific region (Chapman, 1980). Variability in the distribution and diversity of seaweeds is almost identical with phytoplankton productivity in areas close to nutrient-rich up zones (King, 2007). Four major seaweeds floras in sub-Saharan Africa according to Bolton et al. (2003) have been identified, and these include: distinctive

Corresponding authors: **O. Ibraheem**

Tel: +234-81-3942 7707

E. mail: deleibraheem2007@yahoo.com

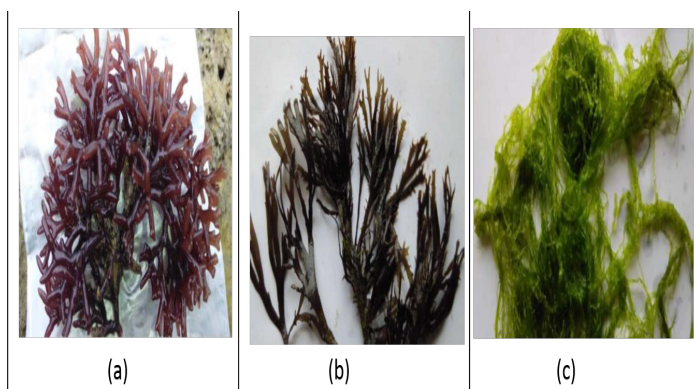
Received: 25-01-2017

Revised: 22-02-2017

Accepted: 15-03-2017

Available online: 01-04-2017

but poor in Tropical West Africa flora; species-rich Tropical East Africa flora that is continuous with much larger Indo-West Pacific flora; cool temperate relatively lower Benguela Marine Province along the West coast of South Africa and the entire Namibian coastline; species-rich warm temperate Agulhas Marine Province along the Eastern and Southern coastlines of South Africa (John and Lawson, 1991). The warm temperate provinces of the Canaries (West African flora to the North) and Namibia are also considered hotspots of seaweeds diversity. So far, about 79 species of seaweeds comprising 38 red, 24 green and 17 brown species have been identified in Nigeria (Algae Base). Nigeria has a coastline of about 860 km exclusive of indentations in the Niger Delta (Solarin et al., 2014).



**Figure 1.** Various classes of seaweeds (a) Red seaweeds; *Gracillaria* spp. (b) Brown seaweeds; *Dictyota ciliolate* (c) Green seaweed; *Enteromorpha clathrata* (Adapted from Madhu et al., 2015).

The ecological and commercial relevance of these marine macroalgae are still unfamiliar with many individuals. Seaweeds are crucial primary producer in oceanic aquatic food webs and have been used as a source of food, though this depends on the species (Solarin et al., 2014). Historically, seaweeds were used in Europe for heating, in mattresses, for feed, and for human food in times of starvation (Dawczynski et al., 2007). Additionally, seaweeds were used to extract soda, hydrocolloids and iodine; a popular disinfectant in the 19th century (Kilinc et al., 2013). The primary uses may have evolved, but some traditional uses remain and are still observed in some coastal areas, for example the use of seaweed for cattle feed and for soil improvement (Sivasankari et al., 2006). Some seaweeds can be eaten as a vegetable, while others are used as fertilizers or processed mainly for the production of hydrocolloids, including carrageenan, agar-agar, and alginate. In country like France, the food-processing;

chemistry and microbiology industries, are the main markets for seaweed, with 75 % of the harvested seaweed (domestic production and imports) used by these sector (McHugh, 2003). Approximately 25% of the seaweed is used in the agricultural, health and well-being sector (López-mosquera et al., 2011).

Some biological component have been isolated from numerous seaweed, which include most especially hydrocolloids (such as alginate, agar, carrageenan, fucoidan) and other biochemical composition such as proteins, lipid, vitamins, minerals and carotenoids (Fleurence, 1999). Alginate tend to be a very important biochemical composition which serve as a resources in a wide range of industrial applications, such as essential components of thickening, gelling or stabilizing agents, and in some cases immunostimulatory agents (McHugh, 1987). It is generally agreed that all species of the brown (Phaeophyceae spp.) seaweeds contain alginates (Gopinathan and Panigrahy, 1983).

According to Dhargalkar and Pereira (2005), the presence of formidable chemical barriers in the structures of these commercially important products creates a serious constraint to their chemical synthesis. Hence, the dependence on seaweeds particularly as a sole source of hydrocolloids is bound to continue in the absence of alternative sources. However the preference of alginate extraction to these bioorganic components owe to its high market value and its peculiar important industrial applications.

Throughout the entire shoreline of West Africa, the seaweeds are highly abundant creating great disturbance and nuisance towards easy access to the coastal shore, consequently many resources and energy are channeled towards their removal yearly (Solarin et al., 2014). This study is therefore geared towards the conversion of this natural nuisance to wealth creation by utilizing the abundant seaweeds towards production of alginate, which could be further exploited for commercial production for various industrial applications.

The developed countries such as China, Australia, Japan and USA are currently been cultivated seaweeds to have more resources for alginate production. On the other hand, Nigeria is yet to explore the enormous wealth abundant in these seaweeds. It is assumed that Nigeria coastal shoreline that significantly has diverse genera seaweeds population could be tapped for commercial exploitation of alginate production. Therefore this study seek to proffer an enabling fast and cheap modular frame work that will facilitate easy extraction of alginate in a fast-time saving process from these seaweeds and also to

determine the biochemical composition. It is expected that these process will produce alginate of good quality and yield and the innate biochemical composition of these seaweeds could be further exploited for other human or animal uses.

## 2. MATERIALS AND METHODS

### 2.1. Reagents

Methanol, chloroform, ethanol, phenol, sulphuric acid, SDS (sodium dodecyl sulphate), distilled water, urea, acetone, BSA (Bovine serum albumin), copper sulphate, sodium hydroxide, calcium chloride, sodium carbonate, hydrochloric acid, formal aldehyde, ammonium oxalate, potassium hydroxide and glucose were all of high analytical grade and were obtained either from BDH Limited Poole England or SIGMA-ALDRICH USA.

### 2.2. Equipments

Oven (Genlab), Bench Top Centrifuge (from Pec Midical USA), water bath (Clifton water bath), UV/VIS spectrophotometer (PG instruments), automated pipette (Microlux) analytical balance (Satorious, Germany), pH- meter (Hanna instrument), Magnetic Stirrer (Genlab) and Murfle Furnace( CM furnace, USA).

### 2.3. Collection and preservation of plant material

Pulverized seaweeds (*Dictyota ciliolate*) were received as kind gift from Naval Holding Ltd/GTE, Naval Holdings Complex, Off Muritala Muhammed Express Way, Asokoro Abuja, Nigeria (Figure 2). It was transported in dark polythene nylon bag, stored in an air tight container and kept in dark cupboard until experimental use at Department of Biochemistry General Laboratory, Faculty of Sciences, Federal University Oye – Ekiti, Nigeria.

### 2.4. Extraction and determination of alginate percent yield from pulverized seaweeds

The extraction protocol was carried out following the methods as described by Haug et al. (1974) with slight modification. About 100 g of pulverized seaweeds was weighed and added into 1 L 2% CaCl<sub>2</sub> solution by stirring in and continuous stirring for about 1 h, in order to hydrate the seaweed and to convert all of the cell

wall alginate into fibrous Ca-alginate, thus conserving its structural integrity. The mixture was later pressed through fine linen Muslin cloth, and residue re-dissolved and stirred continuously in 2 L of distilled water for 2 hours, in order to leach out unwanted plant pigments and other contaminants. The mixture was then filtered through another clean Muslin cloth and residue washed again in 2 L of distilled water.



**Figure 2.** Image of pulverized seaweeds sample kept in polythene nylon bag, from Naval Holdings Ltd/GTE, Asokoro, Abuja, Nigeria.

Residue was collected as previously described above and added into 1 L of 10% Na<sub>2</sub>CO<sub>3</sub>, heated to 40 °C in a water bath with continuous stirring and agitation for 5 h. This alkalization step seeks to convert the fibrous alginate to Na<sup>+</sup>-alginate gel. The gel was then filtered through a doubled muslin cloth and was careful and gently pressed out. Subsequently, 1 M of HCl was added to the filtrate until pH 1.5 was achieved for conversion of Na<sup>+</sup>-alginate back to insoluble alginic acid as a brown floating precipitates which later settle down after standing for 2 h.

The precipitated alginic acid was dehydrated in ethanol at ratio (2:1). The concentrated alginic acid was obtained by aspirating out the clear top ethanol solution, and resultant precipitate completely dried in an oven set at 50 °C. The percent alginate yield was estimated as:

Alginate yield (%) = (weight of dried alginate/weight of dry seaweed) x 100

### 2.5. Extraction and estimation of brown seaweeds biochemical constituents

#### 2.5.1. Protein

This method was carried out according to Shen et al. (2003) with modification using acid washed sand in lieu of alumina in breaking the seaweeds. About 20 g of

pulverized seaweed was homogenized with 40 g of acid washed sand to powdery form by manually grinding in mortar with pestle. 50 mL of 10% SDS sodium dodecyl sulphate was added to 20 g of homogenous sample and placed in water bath at 100 °C for 30 min with occasional stirring, in order to denature the protein. The sample was then centrifuge at 3000 rpm for 10 min and the supernatant removed. Cold acetone was then added to the supernatant at ratio 2:1, placed on ice for about 10 min ensuring precipitation of the protein, and then centrifuged at 3000 rpm for 10 min. The precipitate was later dissolved in 8 M urea solution and vortexed for 3 min to ensure all precipitate was dissolved. About 6 mL of protein solution was obtained and this was used in the estimation of the protein content as described below.

About 50 mg of Bovine serum albumin (BSA) was weighed into a clean test tube and made up to 5 mL with distill water to give a stock of 10mg/ml as the standard BSA solution for protein determination. From the stock BSA solution 60 µL (300 µg protein), 120 µL (600 µg protein), 200 µL (1000 µg), 300 µL (1500 µg), 360 µL (1800 µg protein) and 480 µL (2400 µg protein) were pipette in triplicates into 12 well labeled test tubes (2 tubes per BSA solution), and made up to 2 mL with distilled water. Also 1 mL of extracted protein sample was pipette to 3 separate test tubes and made up with distilled water to 2 mL.

To all test tubes, 3 mL of freshly prepared Biuret reagent was added, vortexed, incubated in dark cupboard at room temperature for 10 min and the absorbance read at 600 nm in UV Spectrophotometer, using distilled water as blank.

The seaweed protein sample concentrations were extrapolated from the Protein (BSA) standard curve where absorbance of BSA standard concentration was plotted against their respective absorbance at 600nm. As analyzes were done in triplicates and results estimated as mean of the triplicate values.

Percent yield of protein in 20 g seaweed =  $(\text{Protein concentration} \times 6 \times 20 \times 100) / (1000 \times 60)$

### 2.5.2. Lipid

The extraction and estimation of the seaweed lipid content was done according to Bligh and Dyer (1959). Chloroform-methanol mixture was prepared by addition of 50 mL of chloroform and 100 mL of methanol in a conical flask and this was used as solvent for the seaweed lipid extraction.

About 10 g of pulverized seaweed was homogenized with 20 g of acid washed sand to powdery form by manually grinding in mortar with pestle. 60 mL

of chloroform- methanol mixture was added to 10 g of homogenous sample in a conical flask and this shaking continuously for 20 min. The resulting solution was centrifuged and clear chloroform-methanol mixture poured into a separating funnel. The methanol upper layer was aspired leaving the lower chloroform - lipid mixture which was further clarified by centrifugation at 3000 rpm for 10 min. This clear chloroform - lipid mixture was poured into a crucible and placed in water bath set at 100 °C until all chloroform evaporates leaving only the extracted oil.

The amount of extracted oil above was quantified as described below:

Weight of crucible = (W1)g

Weight of crucible+chloroform-lipid mixture = (W2)g

Weight of crucible after complete chloroform evaporation, leaving only the oil = (W3)g

Amount of oil in the chloroform-lipid extract =  $(W3 - W1)g$

Percent yield of lipid in 10 g seaweed =  $((W3 - W1) \times 10 \times 100) / 30$

### 2.5.3. Carbohydrate

The carbohydrate content was extracted according to the method as described by Mian and Percival (1973) and estimated by phenol-sulphuric acid method as describe by Dubois et al. (1956). About 15 g of pulverized seaweed was homogenized with 30 g of acid washed sand to powdery form by manually grinding in mortar with pestle. Next, 25 g of homogenous sample was added to 250 mL acetone and stirred for 30 min in order to remove the plant pigment. The mixture was centrifuged and the resulting precipitate collected and oven dry at 50 °C for 24 h in order to dry off the residual acetone. The acetone washed sample was extracted as a hot (70 °C) 80% aqueous ethanol with constant stirring for 1 hour. The sample was centrifuged and precipitate was collected and air dried.

To the resultant precipitate was added to 1 L 2%  $\text{CaCl}_2$  solution and stirred continuously for 1 h, centrifuged and to the precipitate 1 L of 1 M HCl was added, stirred for 30 min, precipitate collected, and this process was repeated 3 times.

To the precipitate, 1 L 10%  $\text{Na}_2\text{CO}_3$  was added and heated to 40 °C in a water bath with continuous stirring and agitation for 5 h. The precipitate was collected and 1 L of 0.25% Ammonium oxalate was added and heated to 70 °C in a water bath with continuous stirring and agitation for 5 h. The precipitate was extensively washed in water by passing water on it through doubled muslin cloth, and 1 L of 6 M KOH was added to the residual sample and stirred

for 5 h at room temperature. The sample was washed by passing water on it through doubled muslin cloth with 1 L each of dilute acetic acid, water, ethanol and ether, in successions.

About 300 mg of extracted sample above was weighed into test tube and solubilized in 5 mL 1 M NaOH. The sample was then centrifuge at 3000 rpm for 10 min and the supernatant collected.

About 100 mg of standard glucose was dissolved in 5 mL of distilled water to prepare glucose stock solution of 20 mg/mL. From this glucose stock solution 0.2 mL (2 mg glucose), 0.4 mL (4 mg glucose), 0.8 mL (8 mg glucose), 1.2 mL (12 mg glucose) and 1.6 mL (16 mg glucose) were pipette into four different test tubes, and made up to 2 mL with distilled water. Also 1 mL of sample was added to a separate test tube and made up to 2 mL with distill water. All these were done in triplicates.

About 4 mL of 80% phenol solution was added to glucose standard solutions and sample and mixed for 1 min. About 2 mL of concentrated  $H_2SO_4$  was then added in stream and allowed to stand for 10 min at room temperature. The solutions were then read at 490 nm using UV spectrophotometer.

The absorbance readings obtained for the standard glucose solutions were plotted against respective glucose concentrations to give Glucose Standard Curve and carbohydrate content of the alginate sample was extrapolated from it. As analyzes were done in triplicates, results are estimated as mean of the triplicate values.

Percent yield of carbohydrate in 300 mg sample =  $(\text{glucose concentration} \times 5 \times 100) / 100$

#### 2.5.4. Total Ash Content

Total ash content was determined according to the method described by Analysis of the Association of Official Analytical Chemists (AAOC, 1990).

To determine the total ash content, an empty crucible was weighed and noted as W1. About 1.0 g of seaweed was weighed into the empty crucible and weight noted as W2. This was transferred into a pre heated muffle furnace at 800 °C for 24 h for ashing. The crucible was cooled down in a desiccator, and weight noted as W3.

% Total ash of seaweed =  $(W_3 - W_1) / (W_2 - W_1) \times 100$

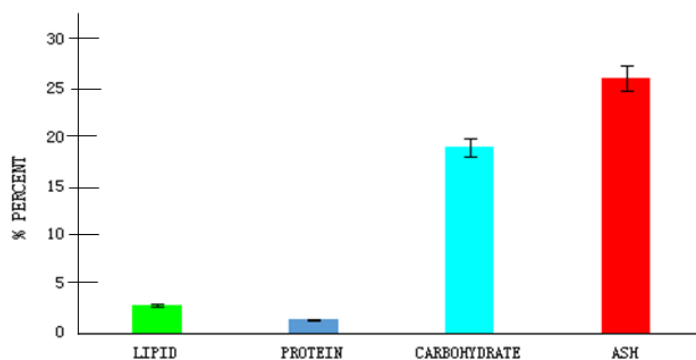
## 3. RESULTS AND DISCUSSION

The alginate obtained from the pulverized seaweed was of light brown in colour (Figure 3) and the percent yield

was 13.34%. The biochemical composition analyzes (Figure 4) showed that the carbohydrate content (19% as estimated by glucose yield); as expected was very higher than the lipid (2.83%) and protein (1.44%) contents. However the ash content (26%) was the highest as this owe to the fact that the bulk plant matter are made up of cellulolytic material that are converted to ash when burnt.



**Figure 3.** Representation of the extracted alginate from pulverized seaweeds of light brown colouration.



**Figure 4.** Biochemical composition of seaweeds. The experiment was performed in triplicates. Values were the averages of means, and represented as Mean  $\pm$  standard deviation. Statistical analyses were performed using student paired T-test. Results were considered statistically significant at  $p \leq 0.05$ .

The percent yield of alginate which was 13.34% and is comparable with what have been previously observed in other research works. As an example, alginate content in *Padina* spp. was estimated to be 16.93% (Mushollaeni, 2011), *S. vulgare* estimated

as 16.9% (Torres et al., 2007), *S. dentifolium*, *S. asperifolium* and *S. latifolium* estimated as 3.25%, 12.4% and 17.7%, respectively (Davis et al., 2003). We presumed that the percent yield could be made higher if only time were taken out to carefully separate the brown seaweeds from other water plant bodies which has no alginate content. However, since they were harvested alongside the brown seaweeds they would have added to the bulk mass of the entire pulverized material that was used in this experiment, consequently the lower yield.

The percent carbohydrate content which was 19% is similar to what was recovered in *U. reticulata*, *T. ornata* and *Sargassum wightii* having 15.37%, 17.49% and 25.5% respectively (Manivannan et al., 2009; Murugaiyan et al., 2012). The carbohydrate content which is higher than 13% alginate recovery may have been the contributions of other polysaccharides in the plant cell.

Though high protein content were previously reported in other work such as 3.25% in green seaweed *U. lactuca*, 17.08% and 7.17% in brown seaweeds *P. gymnospora* and *L. ariegate*, respectively (Manivannan et al., 2008), result obtained however in this work showed a rather low protein concentration of 1.44%. This variation could be attributed to the fact that seaweeds protein contents are largely dependent on seasonal environmental conditions (Dhargalkar et al., 1980; Fleurence, 1999), thus the time of harvesting the seaweeds used in this work, could have played a significant role in the protein content. Furthermore, in many of these reports, liquefied nitrogen was used in breaking the plant tissues and this may have to ensure that the tissues were thoroughly broken, as compared with fine sand that was used in this experiment that may not have broken all the plant tissues, consequently the low protein yield.

Lipid compositions are found to be generally low in many types of seaweed. The percent lipid content in this report which is 2.83% is within the range that has been observed in other works such as in brown seaweeds *Sargassum variegatum* and *P. gymnospora* with 1.0% and 1.4% respectively (Ambreen et al., 2012; Manivannan et al., 2008), green seaweeds *E. clathrata* and *Rhizoclonium implexum* has 4.6% and 8.0% lipid respectively (Manivannan et al., 2008; Ambreen et al., 2012), while red seaweed *G. folifera* has 3.23% lipid content (Manivannan et al., 2008). Drying and pulverizing the seaweeds are factors which are known to contribute to lower lipid content as many polyunsaturated fatty acids are usually lost (Pycke and Faasse, 2015). Seaweeds lipid fractions have shown to

have valuable drug applications, as such we presume that these Nigeria coastal shoreline seaweeds will have many innate pharmacological potentials.

Total ash content obtained was 26% and very similar to that from *P. gymnospora* and *Padina* sp. which were 23.01% and 28.59%, respectively (Mushollaeni, 2011; Shyamala and Thangaraju, 2007). Total ash content reflect majorly the mineral content, thus seaweeds are potential store house of minerals, however the distribution of the minerals essential to human in the different species may vary as a result of hydrology and hydrochemistry of their habitat (Salasa, 2002).

## 4. CONCLUSIONS

Although alginate extraction-production technologies are emerging in many countries such as Norway, Scotland, France, Japan, China and Chile USA, Europe; Africa as a continent is yet to benefit from this, despite the very long coastal shoreline and blessed with abundant seaweeds. This work therefore shows that Nigeria could benefit enormously by commercial production of alginate from these abundant seaweeds for many industrial applications. After thorough biochemical toxicological screenings may have been carried out, the nutritional values of these seaweeds could be exploited for animal feeds, even for human consumption as these seaweeds may house vital essential minerals required for cellular metabolism. Furthermore, the residue plant materials obtained after the alkalization step during alginate extraction process may be exploited as an organic fertilizer since it's basically composed of degradable cellulolytic carbon and coupled with its alkaline nature, it will help to boost the soil fertility and correct soil acidity to the agricultural lands. It could also be exploited as carbon source for lignocellulolytic second generation bioethanol production

In the future, it will be very imperative that the quality assessment of the seaweed alginate extract be done in terms of its purity, intrinsic viscosity, clarity of colour and the ratio of guluronic/mannuronic acid units (G/M) as compared with a commercial available alginate. This will ensure the extracted alginate matches and can be compared favourably with the commercial ones. Nigeria may even be extracting and sell low-grade alginates for further refining to these established Chemical Companies. A kilogram of alginate (low grade) is estimated about US\$ 200; and many overseas companies makes more than \$5 million per annum,

employing many locally (RIRDC, 2014). Thus alginate production will not only create wealth but also provide employments to so many along the Alginate-Business Chain line.

It will also be important in future that the time to be taken in sorting out actual seaweeds from the bulk of harvested sea plant materials, because not all the plant materials may be seaweeds, and adding them to the extraction pool will amount to waste of extraction chemicals and also lead to lower yield; as may have been one of the causes of lower yields observed in this experiment. It is advisable that in future wet harvested seaweeds are processed; as the length of days in drying the seaweeds in open air environment could have encourage microbial contamination, which subsequently will proliferate on the abundant cell wall localized alginate, which may further decrease the alginate yield. Furthermore, comprehensive characterization of the Nigerian coastal shoreline seaweeds should be carried out as composition of alginate in different species differs. The brown seaweeds species are however remarkably known to have very high alginate content. Thus more energy and resources should be channel towards the exploitation of these very species for their suitability for industrial application, equally for conservation and/or subsequent planting of such species as it is done currently in Norway and China.

## ACKNOWLEDGEMENTS

Authors sincerely thank Nigerian Naval Holding Ltd/GTE, Asokoro Abuja, Nigeria, for providing the pulverized seaweed used for this research and Department of Biochemistry, Federal University Oye Ekiti, Nigeria for Laboratory space..

## REFERENCES

- Algae Base, A database of information on algae that includes terrestrial, marine and freshwater organism. www.algaebase.org (Accessed 17<sup>th</sup> October, 2016).
- Ambreen, H.K., Amna, T., Ruqqia, V.S. and Jehan, A. (2012) Evaluation of biochemical component and antimicrobial activity of some seaweeds occurring at karachi coast. *Pakistan Journal of Botany*, 44, 1799-1803.
- AOAC (1990) Official Methods of Analysis of the Association of Official Analytical Chemists, 15th edition Washington, pp. 456-579.
- Bligh, E.G. and Dyer, W.J. (1959) A rapid method for total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911-917.
- Bolton, J.J., Clerck, O. De and John, D.M. (2003) Seaweed diversity patterns in Sub-Saharan Africa. *Proceedings of the Marine Biodiversity in Sub-Saharan Africa: The Known and the Unknown*, 23 - 26 September, Cape Town, South Africa, pp. 229 - 241.
- Chapman, V.D. and Chapman, J. (1980) *Seaweed and Their Uses*. 3<sup>rd</sup> edition. Chapman and Hall. London, pp. 89-95.
- Davis, T., Llanes, F., Volesky, B., Diaz-Pulido, G., McCook, L. and Mucci, A. (2003) <sup>1</sup>H-NMR study of Na alginates extracted from *Sargassum* spp. in relation to metal biosorption. *Applied Biochemistry and Biotechnology*, 110, 75-90.
- Dawczynski, C., Schubert, R. and Jahreis, G. (2007) Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Journal of Food Chemistry*, 103, 891-899.
- Dhargalkar, V.K. and Pereira, N. (2005) Seaweeds: promising plants of the millennium. *Journal of Science and Culture*, 71, 60-6.
- Dhargalkar, V.K., Jagtap, T.G. and Untawale, A.G. (1980) Biochemical constituents of seaweeds along the Maharashtra coast. *Industrial Journal of Marine Science*, 9, 297-299.
- Dubois, M., Giles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Calorimetric method for determination of sugars and related substances. *Analytical Chemistry*, 28, 350-356.
- Fleurence, J. (1999) Seaweed proteins: biochemical nutritional aspects and potential uses. *Trends in Food Science and Technology*, 10, 25-28.
- Gopinathan, C.P. and Panigrahy, R. (1983) Seaweed resources. *CMFRI Bulletin*, 34, 47-51.
- Haug, A., Melsom, S. and Omang, S. (1974) Estimation of heavy metal pollution in two Norwegian fjord areas by analysis of the brown alga *Ascophyllum nodosum*. *Journal of Environmental Pollution*, 7, 179-192.
- John, D.M. and Lawson, G.W. (1991) Littoral Ecosystems Tropical Western Africa. In: Mathieson, A.C. and Nienhuis, D.H. (Eds.). *Intertidal and Littoral Ecosystems of the World*, Elsevier, Amsterdam, pp. 297-322.
- Kılınç, B., Çirik, S., Turan, G., Tekogul, H. and Koru, E. (2013) Seaweeds for food and industrial applications. In: *Food Industry*, INTECH Publisher, Chapter 31, pp. 735-748.
- King, M.G. (2007) *Fisheries biology, assessment, and management*. Second Edition. Blackwell Publishing. Oxford, England, pp. 381.
- López-mosquera, M.E., Fernández-lema, E., Villares, R., Corral, R., Alonso, B., and Blanc, C. (2011) Composting fish waste and seaweed to produce a fertilizer for use in organic agriculture. *Procedia Environmental Sciences*, 9, 113-117.
- Madhu, B.K., Lidya, M., Pasience, P.M. and Ghebray, A. (2015) A review on biochemical composition and nutritional aspects of seaweeds. *Caribbean Journal of Science and Technology*, 3, 789-797.
- Manivannan, K., Thirumaran, G., Devi, K.G., Anantharaman, P. and Balasubramanian, T. (2009) Proximate composition of different group of seaweeds from Vedalai coastal waters (Gulf of Mannar): Southeast coast of India. *Middle-East Journal of Scientific Research*, 4, 72-77.
- Manivannan, K., Thirumaran, G., Devi, K.G., Anantharaman, P. and Balasubramanian, T. (2008) Biochemical composition of seaweeds from mandapam coastal regions along the southeast coast of India. *American-Eurasian Journal of Botany*, 1, 32-37.
- McHugh, D.J. (2003) A guide to the seaweed industry. *FAO Fisheries Technical Paper No. 441*, pp. 105 (<ftp://ftp.fao.org/docrep/fao/006/y4765e/y4765e00.pdf>).
- McHugh, D.J. (1987) Production, properties and uses of alginates. *FAO Fish Technology*, 288, 58-115.

- Mian, J. and Percival, E. (1973) Carbohydrates of brown seaweeds. *Carbohydrate Research*, 26, 133-146.
- Murugaiyan, K., Narasimman, S., Anatharaman, P. (2012) Proximate composition of marine macro algae from Seeniappa Dharka, Gulf of Mannar region, Tamil Nadu. *International Journal of Research in Marine Sciences*, 1, 1-3.
- Mushollaeni, W. (2011) The physicochemical characteristics of sodium alginate from Indonesian brown seaweeds. *African Journal of Food Sciences*, 5, 349 – 352.
- Pycke, B.F. and Faasse, M. (2015) Biochemical composition and quality assessment of native macroalgae collected along the Flemish coast. Public output report of the EnAlgae project, Oostende, December 2015, pp. 30.
- RIRDC (2014) Rural Industries, Research and Development Corporation, Australian Government, Emerging Animal and Plant Industries: Their value to Australia, Publication No: 14/069 <https://rirdc.infoservices.com.au/downloads/14-069>
- Salasa, F.F.A. (2002) Seaweeds and Fish Processing Technology. Fisheries Department of Indonesia. Jakarta, pp. 12.
- Shen, S.H., Jing, Y.X. and Kuang, T.Y. (2003) Proteomics approach to identify wound-response related proteins from rice leaf sheath. *Journal of Proteomics*, 3, 527-535.
- Shyamala, V. and Thangaraju, N. (2007) Extraction of Sodium Alginate from Selected Seaweeds and Their Physiochemical and Biochemical Properties. *International Journal of Innovative Research in Science, Engineering and Technology*, 3, 3297.
- Sivasankari, S., Venkatesalu, V., Anantharaj, M. and Chandrasekaran, M. (2006) Effect of seaweed extracts on the growth and biochemical constituents of *Vigna sinensis*. *Journal of Bioresource Technology*, 97, 1745-1751.
- Solarin, B.B., Bolaji, D.A., Fakayode, O.S. and Akinnigbagbe, R.O. (2014) Impacts of an invasive seaweed *Sargassum hystrix* var. *fluitans* (Børgesen 1914) on the fisheries and other economic implications for the Nigerian coastal waters. *IOSR Journal of Agriculture and Veterinary Science*, 7, 1-6.
- Torres, M.R., Sousa, A.P.A., Filho, E.A.T.S., Melo, D.F., Feitosa, J.P.A., Paula, R.C.M. and Lima, M. G.S. (2007) Extraction and physicochemical characterization of *Sargassum vulgare* alginate from Brazil. *Carbohydrate Research*, 342, 2067–2074.