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# Functional characteristics and anti-oxidative potentials of alginate and fucoidan in *Sargassum fluitans* found in Creek Town, Calabar

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#### ABSTRACT

Seaweeds are the largest and most complex marine algae, which are taxonomically classified under three groups namely: brown algae, red algae and green algae. Sargassum fluitans is a genus of brown macro-algae, which are always found in abundance, floating on the sea. In this study, fucoidan and alginate were extracted from Sargassum fluitans, and their anti-oxidant and functional qualities were determined. Fucoidan and alginate extraction was carried out using hot Mcllvains buffer at 60 °C. The test parameters monitored were the anti-oxidant activity (DPPH scavenging activity, hydroxyl radical scavenging activity, metal chelating and reducing power) and functional properties (water holding capacity, water swelling capacity, bulk density and water solubility index). The fucoidan and alginate yield from Sargassum fluitans was 11.12% and 13.11% respectively. The antioxidant properties (IC<sub>50</sub>) of fucoidan were compared to ascorbic acid and gallic acid as standards. The results of the functional properties of water holding capacity, oil holding capacity, water swelling capacity, emulsifying activity and bulk density of fucoidan and alginate are (1.153±0.10 g/g and 2.65±0.43), (1.67±0.25 and  $2.11\pm0.34$ ), ( $2.73\pm0.12$  only for fucoidan), (80% and 89%), and ( $10\pm0.0.00\pm0.62\pm0.00$ ) respectively. The IC<sub>50</sub> of fucoidan and alginate for hydrogen peroxide scavenging, metal chelating, hydroxyl radical scavenging, reducing power, and DPPH scavenging activities were (0.056±0.00 and 0.57±0.00), (0.069±0.00 and 0.081±0.00), (0.075±0.00 and 0.058±0.00), (0.101±0.00 and 0.078±0.04), and (0.058±0.00 and 0.073±0.00) respectively. Data from this study was carried out in triplicate and expressed as Mean  $\pm$  SD, A *p*-value < 0.05 was considered significant. Results from the anti-oxidant activities of the extracts showed the following; the hydrogen peroxide scavenging activity of alginate and fucoidan is greater than that of ascorbic acid, the metal chelating activity of gallic acid is higher than fucoidan and alginate, the DPPH scavenging activity of fucoidan is greater than that of ascorbic acid. It was evident that fucoidan and alginate could serve as functional food source due to their functional properties and could serve as potential antioxidant sources.

Keywords: Sargassum fluitans, Functional properties, Fucoidan, Alginate, Antioxidant

#### **1. INTRODUCTION**

The brown algae are a rich source of bioactive molecules such as proteins, amino acids, polysaccharides, fatty acids, vitamins, minerals, dietary fibre, sterols, pigments, and polyphenols which possess a broad spectrum of biological activities (anticoagulant, antithrombotic, anti-viral, anti-cancer, anti-inflammatory and antibacterial) [33]. These compounds therefore provide high potential for the application of brown algae extracts in the treatment of arteriosclerosis, rheumatic processes, hypertension, goitre, asthma, ulcers, menstrual disorders, syphilis, and skin diseases amongst others [6, 23]. The biological potential of brown algae is considerably contributed by polysaccharides as one of the most common and essential groups of bioactive compounds [26].

*Sargassum fluitans* the brown algae are marine algae which is commonly called the "Gulf weed". *Sargassum fluitans* belongs to the class of *Phaeophyceae*, family of *Sargassaceae* and order of *Fucales* [21].For the past years, substantial interests has been raised about various types of polysaccharides in brown algae cell walls, including fucoidans, alginates and laminarins which have high potential for biological applications in functional foods, cosmetics and pharmaceutical products [13, 27]. The structure and composition of algal polysaccharides (APS) is determined by algae species, however it is also influenced by other factors causing inter-species variation, such as growth location and harvesting season [13]. There is vast structural variation between the algal polysaccharides, this presents a challenge in terms of pre-treatments application, extraction techniques and optimization, characterization of isolated fractions and determination of their functional properties [6].

Fucoidans are recognized as fucose-containing sulfated polysaccharides (FCSPs), where L-fucose mostly predominates other sugar monomers, such as galactose, mannose, glucose, and uronic acids. L-fucose may exceed 90% of the total sugar composition of fucoidans [13]. Yet, galactose, as in the case of sulfated galactofucans, may possess similar ratios to fucose [12]. Another type of fucose-containing sulfated polysaccharides(FCSP) is isolated from marine invertebrates, called sulfated fucans. In contrast, they are composed of L-fucose only [12]. Hence, the term fucoidans has recently been adopted specifically for the heterogenous marine sulphated polysaccharides rich in fucose and derived from the various species of brown algae, including the old names fucoidin and fucoidan to be consistent with the International Union of

Pure and Applied Chemistry (IUPAC) nomenclature system [2]. Various applications of fucoidans have been reported such as therapeutic [17], cosmeceutical, nutraceutical/functional foods [15, 32], diagnostic, and drug delivery [6]. Several fields have increased awareness concerning their importance, especially in the last few decades. Specifically, the pharmacological activities of fucoidans make them a candidate for the treatment of bleeding disorders, inflammation [20], viral infections, malignant tumours [3], and immune disorders. Recently, scientists are focusing on pharmacokinetic and tissue distribution investigations after oral and topical administration of fucoidans [6, 26]. These studies may support the

administration of fucoidans in prospective human clinical trials. Meanwhile, the adjustment of native fucoidans via chemical or enzymatic treatment may result in an increase in their biological activity [24].

Alginates on the other hand, are polysaccharides mostly found in the cellular wall matrix of brown seaweeds (*Pheophyceae*) to which *Sargassum fluitans* belongs and are composed of  $\beta$ -(1 $\rightarrow$ 4)-D-mannuronic (M) and  $\alpha$ -L-guluronic acid (G) blocks in heterogeneous proportions [19]. The polysaccharide is existing in the cell matrix as insoluble calcium or magnesium salt, which makes the tissue flexible and robust [4]. It is predominant in brown seaweed. Alginate has vast industrial applications, it is widely used as an emulsifier, thickener and stabilizing agent in ice cream, toothpaste, mayonnaise industry, for whey separation [6], in milk industries, microencapsulator in medical science, and as microsphere vectors for drug delivery [19]. Among other functions, alginate shows anti-cancer activities and pre-biotic properties that promote health [16], strong free radical scavenging activity has also been reported, and renoprotective effect. Solvent extracts of the brown seaweed species *Sargassum muticum* have shown antioxidative and antimicrobial activities and used as natural preservatives [16]. Considering the vast applications of these essential polysaccharides and their functional properties, this research is focused on extraction and functional characterization of fucoidans and alginate in the brown seaweed *Sargassum fluitans*.

Due to the underutilization of *Sargassum* in Nigeria majorly due to the paucity of/low scientific studies that helps validate the biological importance of *Sargassum*, it is seen as a pollutant. The aim of this study is to determine the functional properties and Antioxidant potentials of fucoidan and alginate extracted from *Sargassum fluitans* using standard scientific methods.

#### 2. RESULT

#### 2. 1. Extraction of Fucoidan

| Extract  | Mass of Sargassum (g) | Mass of fucoidan obtained (g) | Yield (%) |
|----------|-----------------------|-------------------------------|-----------|
| Fucoidan | 500                   | 55.61                         | 11.12     |

The Table 1 showed that 500g of *Sargassum fluitans* gave 55.61g of fucoidan whose yield is calculated as 11.12%.

#### 2. 2. Extraction of Alginate

| Extract  | Mass of Sargassum | Mass of Alginate (g) | Yield (%) |
|----------|-------------------|----------------------|-----------|
|          | ( <b>g</b> )      |                      |           |
| Alginate | 500g              | 65.57g               | 13.11     |

Table 2. The percentage yield (%) of the Alginate.

The Table 2 showed the percentage yield of alginate from 500g of *Sargassum fluitans*, 65.57g was gotten with a yield of 13.11%.

# 2. 3. Functional Properties of *Sargassum fluitans* in Comparison to Isolated Polysaccharides, Fucoidan and Alginate

|           | WHC<br>(g/g)  | OHC<br>(g/g)   | SWC<br>(ml/g) | EA<br>(%) | Bulk Density<br>(g/ml) |
|-----------|---------------|----------------|---------------|-----------|------------------------|
| Sargassum | $6.45\pm0.03$ | $2.39\pm0.007$ | 7.33 ± 0.133  | 60        | 1.429                  |
| Fucoidan  | 1.153 ±0.101  | 1.67 ±0.250    | 2.733 ±0.115  | 80        | 1.0 ±0.000             |
| Alginate  | 2.65±0.43     | 2.11±0.34      |               | 89        | 0.624±0.0012           |

Table 3. Functional Properties of Sargassum fluitans at 25 °C

The Table 3 showed the functional properties of *Sargassum fluitans* is higher compared to alginate and fucoidan extracts of *Sargassum fluitans*.



Figure 1. Water Holding Capacity

As shown in Figure 1, *Sargassum fluitans* has a peak water holding capacity of 6.45g/g at room temperature. The water holding capacity then drops significantly at 40 °C and later stabilizes at 60 °C through 80 °C.



Figure 2. Oil Holding Capacity

Figure 2 showed the oil holding capacity of *Sargassum fluitans* in comparison withalginate and fucoidan at different temperatures. The *Sargassum fluitans* shows consistent increase in oil holding capacity following increase in temperature, having a value of 2.38g/g at room temperature and 4.63g/g at 80 °C.



Figure 3. Water Swelling Capacity

Figure 3 showed the WSC of sargassum in comparison to fucoidan and alginate. The WSCs of alginate were substantially higher than that of fucoidan and Sargassum.



EMULSIFYING ACTIVITY

Figure 4. Emulsifying Activity

Figure 4 showed the emulsifying activity of *Sargassum fluitans*in comparison to fucoidan and alginate. The emulsifying activity of alginate were substantially higher (89%) than that of fucoidan and *Sargassum*.

| IC50 (mg/2    | ml)  |                               |   |                           |                             |
|---------------|--|-------------------------------|---|---------------------------|-----------------------------|
| EXTRACTS      | HYDROGEN<br>PEROXIDE<br>SCAVENGING<br>ACTIVITY | METAL<br>CHELATING<br>ABILITY | HYDROXYL<br>RADICAL<br>SCAVENGING<br>ACTIVITY | REDUCING<br>POWER         | DPPH SCAVENGING<br>ACTIVITY |
| Fucoidan      | $0.056\pm0.001\text{a}$                        | $0.069 \pm 0.003a$            | $0.075\pm0.001b$                              | $0.101\pm0.001b$          | $0.058\pm0.003a$            |
| Alginate      | $0.057\pm0.002a$                               | $0.081 \pm 0.001 b \\$        | $0.058\pm0.001a$                              | $0.078 \pm 0.040 a$       | $0.073\pm0.003b$            |
| Ascorbic Acid | $0.131\pm0.010b$                               |                               | $0.056\pm0.008a$                              | $0.063\pm0.001 \text{ac}$ | 0.161±0.010c                |
| Gallic Acid   |  | $0.059 \pm 0.001 \mathrm{ac}$ |   |                           |                             |

Values are represented as Mean  $\pm$  SEM. Values with different alphabets are statistically different from each other at p $\leq$ 0.05 within each column.



Figure 5. Hydrogen peroxide scavenging activity

Figure 5 showed the hydrogen peroxide scavenging activity of Alginate, compared with ascorbic acid as standard, the activity of alginate and fucoidan show a concentration independent activity while ascorbic acid shows a concentration dependent activity. The hydrogen peroxide scavenging activity of alginate and fucoidan is greater than that of ascorbic acid.



Figure 6. .Metal Chelating Ability

Figure 6 showed the metal chelating ability of Fucoidan and alginate, compared with Gallic acid as the standard, all showed a concentration independent activity, exception at 0.2 mg/ml

which exhibited the highest metal chelating ability of Fucoidan. The metal chelating activity of gallic acid is higher than fucoidan and alginate.



HYDROXYL RADICAL SCAVENGING ACTIVITY

Figure 7. Hydroxyl Radical Scavenging Activity

Figure 7 showed the hydroxyl radical scavenging activity of fucoidan and alginate, compared with Ascorbic acid as the standard. The hydroxyl radical scavenging activity of fucoidan was exhibited in a concentration dependent manner, showing 0.1 mg/ml with the highest scavenging activity, there is a significant difference in the activity of fucoidan and alginate to ascorbic acid.



Figure 8. Reducing Power

Figure 8 showed the reducing power activity of alginate and fucoidan compared with ascorbic acid as the standard. The 0.1 mg/ml and 0.2 mg/ml concentration of the fucoidan and alginate respectively exhibited the highest scavenging activity while the 0.4 mg/ml and 0.5 mg/ml of alginate and fucoidan exhibited the lowest scavenging activity respectively. The

reducing power activity of alginate at 0.1 mg/ml is lesser than that of ascorbic acid. There is a significant difference between the activity of alginate and fucoidan to ascorbic acid at 0.2-0.5 mg/ml, indicating that the reducing power activity is greater at only those concentrations.



Figure 9. DPPH Scavenging Activity

Figure 9 showed the DPPH scavenging activity of fucoidan and alginate compared with ascorbic acid as standard, the activity of fucoidan and alginate showed a concentration independent activity while ascorbic acid shows a concentration dependent activity. The 0.1 mg/ml and 0.2 mg/ml concentration of the alginate and fucoidan respectively exhibited the highest scavenging activity while the 0.3 mg/ml and 0.4 mg/ml exhibited the lowest scavenging activity for fucoidan and alginate respectively, there is significant difference between the activity of fucoidan and ascorbic acid. The DPPH scavenging activity of fucoidan is greater than that of ascorbic acid.

#### 3. EXPERIMENTAL DESIGN

#### 3.1. Materials

All the equipments used in this study were of laboratory standard. These include the following: Beakers, Water bath, Hot plate, Weighing balance, Measuring cylinders, Thermometers, Plastic buckets, Stirrer, Stop watch, Centrifuge machine, Chromatography, Plates, Test tubes, Test tube holder spatula, Blender, Microsyringe filter, Refrigerator, pH meter, Fluorescence spectrophotometer, Funnel, Oven, Whatman filter paper.

All chemicals used in this study were of analytical grade and they include the following: Distilled water, HCl, Ethanol, Sodium sulphate standard, Ascorbic acid, NaCl, CaCl<sub>2</sub>, Na<sub>2</sub>CO<sub>3</sub>, L-fucose standard, Phosphate buffer standard, DEAE sephadex A-25.

#### 3. 2. Sample Collection and Identification

Fresh brown seaweed (*Sargassum fluitans*) were collected from Creek Town, a coastal area of Calabar, Cross River State, Nigeria. After collection, the seaweed was taken to the

Laboratory kept in plastic bags with sea water. The seaweed was authenticated by an expert in marine organisms in the Botany Department of University of Calabar. Thereafter, the samples were washed with tap water and rinsed with distilled water, and allowed to dry at room temperature for 7 days. The *Sargassum fluitans* were pulverized into coarse powder using grinding machine [11].

#### 3. 3. Extraction of Fucoidan from Sargassum fluitans using Alcohol

The extraction of fucoidan was carried out using alcohol [28], it is one of the standard methods. Briefly, the milled sample were mixed with 95% ethanol in the ratio (1:2) and shaken thoroughly for 1 hour to remove pigments, proteins and lipids and was centrifuge for 10 minutes. The precipitate was collected, mixed with double distilled water (w/v=1:10) and placed in a water bath maintained at 40 °C for 15 minutes with shaking. The mixture was centrifuged at 3870g for 10 minutes and the supernatant was collected. Ethanol (95%) was added to the collected supernatant to give a final ethanol concentration of 20%. The mixture was then centrifuged at 9170g for 30 minutes, the supernatant was collected and 95% ethanol was added to a final ethanol concentration of 50% and fucoidan was obtained. The precipitated fucoidan was collected by centrifugation at 9170g for 30 minutes and was dried at 40 °C.

#### 3. 4. Calculation of Fucoidan Yield

Yield of Fucoidan (%) = 
$$\frac{\text{Weight of fucoidan (g)}}{\text{Weight of milled seaweed (g)}} \times 100$$

#### 3. 5. Purification of Fucoidan from Sargassum fluitans

The purification of fucoidan was conducted using the DEAE Sephadex A-25 and eluted by gradient NaCl step wise elution as described by Duarte *et al.* [9]. Briefly, crude polysaccharide (Fucoidan extract) was dissolved in 0.1 M buffer Tris-Cl pH 7.0 was loaded to a column of DEAE Sephadex A-25 with a fast flow rate of (4 cm  $\times$  25 cm), then followed by step-wise elution using 50 ml of sodium chloride solutions (0.5 – 2.5 M) at the flow rate of 1 ml/min. Eluents (5 mL/tube) were collected separately and ethanol (HPLC grade) were added with ratio 1:2. All fractions were filtered, freeze-dried and stored at 4 °C till used. The carbohydrate content in each eluent was determined using a phenol-sulphuric acid method [10] using commercial fucose as standard. This is the most widely used colorimetric method to date for determination of carbohydrate concentration in aqueous solutions [10].

The basic principle of this method is that carbohydrates, when dehydrated by reaction with concentrated sulfuric acid, produce furfural derivatives. Further reaction between furfural derivatives and phenol develops detectible colour. The standard procedure of this method is as follows. A 2mL aliquot of a carbohydrate solution was mixed with 1 mL of 5% aqueous solution of phenol in a test tube. Subsequently, 5 mL of concentrated sulfuric acid was added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they are vortexed for 30 s and placed for 20 min in a water bath at room temperature for colour development. Then, light absorption at 490 nm was recorded on a spectrophotometer. Reference solutions were prepared in identical manner as above, except that the 2 mL aliquot of carbohydrate was replaced by deionized water. The phenol used in this procedure was redistilled and 5% phenol in water (w/w) was prepared immediately before the measurements.

#### 3. 6. Extraction of Alginate from Sargassum fluitans

Alginate was extracted by cold method as earlier reported by [5]. Briefly, 20 g of airdried seaweed samples were soaked in 300 mL of 1% CaCl<sub>2</sub> solution at roomtemperature (27 °C) overnight (around 18 hours). Then, the seaweed was washed with distilled water (3×300 mL), stored in 5% HCl solution for 1 hour and washed again with distilled water (3×300 mL). After that, the samples were stored in 300mL of 3% Na<sub>2</sub>CO<sub>3</sub> solution for 1 hour and250 mL of water was added into it before it was left to stand overnight. The viscous mixture was separated from its residue by centrifuging at 14,000×g. Sodium alginate extracted was precipitated from the solution by adding an ethanol/water mixture (1:1, v/v). The precipitate was filtered, washed with ethanol again and dried in air, followed by drying in a vacuum oven. Yield of alginate can be calculated as follows:

### 3. 7. Calculation of Alginate Yield

Yield of Alginatyte (%) = 
$$\frac{\text{Weight of alginate (g)}}{\text{Weight of dry seaweed (g)}} \times 100$$

#### 3. 8. Characterization of the Functional Properties of Sargassum fluitans

#### 3. 8. 1. Swelling Capacity (SWC)

SWC of seaweed samples was analyzed by the bed volume technique after equilibrating in excess solvent [7]. Briefly, to 200 mg of seaweed samples in a 50 ml measuring cylinder, 20 ml of de-ionized water were added and the mixture was vigorously stirred, poured into the measuring cylinder and was allowed to stand for 24h at 25 and 37 °C. Swelling volume was measured and expressed as milliliters of swollen sample per gram of sample (DW).

#### **3. 8. 2. Water Holding Capacity (WHC)**

WHC of seaweed samples was measured by the modified centrifugation method described by Suzuki *et al.* [29]. Briefly, 20 ml of de-ionized water were added to each centrifuged tube containing 200 mg of seaweed samples and the mixture was vigorously stirred. The tubes were left to stand for 24h at 25and 37 °C and were centrifuged at 14,000 ×g for 30 min, the supernatant was discarded and the moisture content of pellet were determined after dehydration in an oven for 2 h at 120 °C. The WHC of seaweed was expressed as the weight of grams of water held by 1 g of sample (DW).

#### 3. 8. 3. Oil Holding Capacity (OHC)

Oil Holding Capacity (OHC) was calculated according to Chakraborty with slight modifications. 2 g of *Sargassum fluitans* were weighed in plastic centrifuge tube. For each sample, 20 ml of sunflower was added and well mixed with the sample using a vortex mixer at the highest speed, the samples will be subsequently allowed to stand at room temperature ( $22\pm2$  °C) for 30 min. Sample-oil mixture was centrifuged at 1200g for 30 min, the supernatant was carefully decanted and the new mass of the sample were calculated as

 $OHC = (M_{oiled} - M_d) / M_d$ 

Where,  $M_d$  and  $M_{oiled}$  are the mass of dry material and the mass of the sample including the oil, respectively.

#### 3.8.4. Foaming properties

Foaming capacity (FC) and foam stability (FS) were determined according to the method of Makri *et al.* [18]. Foam capacity (FC) was measured in terms of volume increase on whipping expressed as percentage of original volume of the liquid. Foam stability (FS) was expressed as percentage of foam volume remaining, in relation to initial foam volume at room temperature  $(25\pm 2^{\circ}C)$  after 5, 10, 20, 30, 40, 50, 60, 90 and 120 min.

Foaming capacity (%) =  $\frac{\text{Vol. after homogenization} - \text{Vol. before homogenization}}{\text{Vol. before homogenization}} \times 100$ 

#### 3.8.5. Gelation properties

Gelation properties of the sample were determined by employing the method of Adebowale *et al.* [1]. Sample suspensions of 2 to 20% (w/v) were prepared in 5 ml distilled water. The test

Foam stability (%) = 
$$\frac{Foam \ volume \ after \ time \ (t)}{Initial foam \ volume} \times 100$$

tubes containing these suspensions were heated for 1 h in boiling water (100 °C), followed by rapid cooling in ice. The test tubes were then cool for 24 h at 4 °C. The least gelation concentration (LGC) was seen as the concentration when the sample from inverted test tube did not fall down or slip.

#### 3.8.6. Bulk Density

The volume of 100 g of the sample was measured in a measuring cylinder (250 ml) after tapping the cylinder on a wooden plank until no visible decrease in volume was noticed, and based on the weight and volume and the apparent (bulk) density was calculated [14].

#### 3.8.7. Emulsion

The emulsion activity and stability by Yasumatsu *et al.* [31] was followed. Briefly, 1 g sample, 10 ml distilled water and 10 ml soybean oil wasprepared in calibrated centrifuged tube. The emulsion was centrifuged at  $2000 \times g$  for 5 min. The ratio of the height of emulsion layer to the total height of the mixture was calculated as emulsion activity in percentage. The emulsion stability was estimated after heating the emulsion contained in calibrated centrifuged tube at 80 °C for 30 min in a water-bath, cooling for 15 min under running tap water and centrifuging at 2000  $\times$  g for 15 min. The emulsion stability expressed as percentage was calculated as the ratio of the height of emulsified layer to the total height of the mixture.

# 3. 9. In vitro Antioxidant Activity of Seaweed

### 3. 9. 1. Total Antioxidant Activity

Total antioxidant activities of crude ethanol and acetone extract obtained from seaweeds was determined following standard methods of Prieto *et al.* [25]. Briefly, 0.3 ml of sample was mixed with 3.0ml reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95°C for 90 minutes under water bath. Absorbance of all the sample mixtures was measured at 695nm. Total antioxidant activity was expressed as the number of equivalents of ascorbic acid in milligram per gram of extract.

#### 3.9.2. Reducing Power

Reducing power of crude ethanolic and acetone extract obtained from seaweeds was estimated following standard methods of Oyaizu [22]. Briefly, 1.0 ml of methanol containing different concentration of sample with be mixed with 2.5 ml of phosphate buffer (0.2M, pH-6.6) and 2.5 ml potassium ferricyanide (1%). Reaction mixture was incubated at 50°C for 20min. After incubation 2.5 ml of trichloroacetic acid (10%) was added and centrifuged (650 g) for 10min. From the upper layer 2.5 ml solution was mixed with 2.5 ml distilled water and 0.5 ml FeCl3 (0.1%). Absorbance of all the sample solutions was measured at 700nm. Increased absorbance indicates increased reducing power.

### 3. 9. 3. DPPH (2, 2- diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The scavenging effects of ethanol and acetone extract obtained from seaweeds were determined [30]. Briefly, 2.0 ml of 0.16mM DPPH solution (in methanol) was employed to the test tube containing 2.0 ml aliquot of sample. The mixture was vortexed for 1min and kept at room temperature for 30 min in the dark. The absorbance of all the sample solutions was measured at 517nm. The scavenging effect (%) was calculated by using the formulae [8]. Sample blank and control samples were performed according to the method. Scavenging effect of DPPH radical was calculated using the following equation:

DPPH radical scavenging activity [%] =  $[1 - (A_{sample} - A_{sample \ blank} / A_{control}) \times 100]$ 

where  $A_{sample}$  is the Absorbance of DPPH solution & test sample,  $A_{sample \ blank}$  is the absorbance of the sample only without DPPH solution). Synthetic antioxidant Ascorbic acid was used as positive controls.

#### **3. 10. Statistical Analysis**

Data from this study was carried out in triplicate and expressed as Mean  $\pm$  SD, using SPSS version 23, followed by One-way Analysis of variance (ANOVA) to ascertain the level of statistical significance among the test parameters. A *p*-value < 0.05 was considered significant.

# 4. CONCLUSIONS

This study was designed to determine the functional properties of fucoidan and alginate in *Sargassum fluitans*. he study findings revealed percentage yield of alginate to be higher than

fucoidan in *S. fluitans* this is in line with the reports of Mazumder *et al.* [19]. In this current study the water holding capacity of the Alginate was relatively higher (2.65 g/g) than fucoidan while *S. fluitans* recorded highest (6.45 g/g) water holding capacity at room temperature. The differences noted in the seaweed samples with temperature in WHC may be due to protein conformations and the changes in the number and nature of the protein molecules' water-binding sites. In this current study the Oil Holding Capacity (OHC) of *S. fluitans* at room temperature was lower than that at higher temperatures  $(4.63 \text{ g/g} at 80 \,^{\circ}\text{C})$  which is a desirable attribute in the food industry.

The water swelling capacity of the functional alginate gotten from *S.fluitans*, was significantly higher (2.65 g/g) than *S. hemiphyllum*, which indicated that alginate from *S.fluitans* would be more suitable for being used a as texturizing and bulking agents in making low calories food products that have a greater potential to be used as a functional ingredients. The alginate extracted from *Sargassum fluitans* was significantly lower (0.624 g/ml) than the bulk density of wheat flour. In this study the emulsifying activity of alginate was substantially higher (89%) than fucoidan and *Sargassum fluitans*. The high activity of this emulsion can be applied in the manufacturing industries for the production of soaps, antiseptics and disinfectants and as well as in the production of medicines.

In this current study, the alginate and fucoidan extracts of *Sargassum spp*, exhibited high inhibitory activity in hydrogen peroxide scavenging when compared with ascorbic acid (Figure 5). Hydroxyl radical scavenging activity is an assay used to examine if a natural compound has an antioxidant effect in mobbing up free hydroxyl molecule. In this study fucoidan showed its highest inhibition at 0.1 mg/mL whose percentage scavenging activity is 77.00 $\pm$ 2.00% and an IC<sub>50</sub> value of 0.075 $\pm$ 0.0001 mg/mL, closely followed by alginate (Figure 7). The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) scavenging activity is a method used to examine if a natural compound has antioxidant activity in which it reduces DPPH to DPPH-H, which changes the original colour of DPPH purple to yellow. In this study fucoidan showed its highest inhibition at 0.2 mg/mL whose percentage scavenging activity is 93.67 $\pm$ 0.97% and an IC<sub>50</sub> value of 0.058 $\pm$ 0.005 mg/mL (Figure 9). It is evident that the extracted alginate and fucoidan from *Sargassum fluitans* showed potent antioxidant activity and could be applied in the pharmaceutical and nutraceutical industries. The extracted functional properties alginate and fucoidan from *Sargassum fluitans* exhibited wide range of industrial applications from cosmetics, food, paint, and pharmaceutical industries.

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