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Abdulatif Olufemi Giwa-Ajeniya
Department of Chemistry, Lagos State University, Ojo, Lagos, Nigeria

Ayobami Oluwatimilehin Olatayo
Department of Chemistry, Lagos State University, Ojo, Lagos, Nigeria

Isiaka Ajani Ogunwande
Foresight Institute of Research and Translation, Science and Technology Section, Eleyele-Ologuneru Road, Ibadan, Oyo State, Nigeria

Corresponding Author:
Abdulatif Olufemi Giwa-Ajeniya
Department of Chemistry, Lagos State University, Ojo, Lagos, Nigeria

Phytochemical compositions, volatile constituents, antioxidants and antidiabetic activities of *Sargassum natans* (Linn.) Gaillon from Nigeria

Abdulatif Olufemi Giwa-Ajeniya, Ayobami Oluwatimilehin Olatayo and Isiaka Ajani Ogunwande

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Abstract
Sargassum natans is a specie of brown seaweed belonging to the family Sargassaceae. It can be processed into food, pharmaceutical products, cosmetics and textiles. Besides having economic value and protection for marine biota, it is beneficial in the health field. This research was aimed at investigating the phytochemical screening, volatile chemical compositions, antioxidant and antidiabetic activities of the methanolic extract of *S. natans*. The antioxidant activity was tested by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. The antidiabetic potential was assessed by evaluating the inhibitory effect of the extract on the activities of α-amylase and α-glucosidase enzymes. The volatile compositions were identified using Gas chromatography-mass spectrometry (GC-MS) analysis. The phytochemical screening showed the abundance of saponin (38.77 mg/100g), alkaloid (26.55 mg/100g), cardiac glycoside (25.45 mg/100g) and phlobatannin (21.05 mg/100g) contents. In addition, at 100 µg/mL, *S. natans* exhibited 78%, 64% and 65% antioxidant, α-amylase and α-glucosidase inhibitory activities. The main volatile compounds identified in *S. natans* were di-tert-butylphenol (58.04%), palmitic acid (24.65%), and n-nonadecanol-1 (5.98%). *S. natans* can be assumed to contains phytochemicals with promising antidiabetic and antioxidant activities which can serve as a source of future lead drugs for combating oxidative stress related diseases.

Keywords: *Sargassum natans*, phytochemical, antioxidant, antidiabetic, volatile compounds

1. Introduction

Sargassum (Family Sargassaceae) is a genus of brown algae commonly known as gulfweed oor sea holly, and is considered one of the most complex Phaeophyceae genera. *Sargassum* contains about 537 species [1]. This family of plants are known as sources of biologically active compounds. For example, tetraol, fucosterol, linoleic acid, heptadecane, 2,6,10,14-tetramethyl-hexadecane, nonadecane, heneicosane and 4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2[4H]-one were isolated from *S. subrepandum*[2]. *Sargassum* is the largest biological source of open ocean polyphenols such as phlorotannins and their oxygenated phenolic derivatives recorded to date [3]. *Sargassum* seaweed are a source of anti-inflammatory [4], antioxidant, antibacterial [5] and other pharmacologically and natural products [1]. *S. dentifolium* extract demonstrated significant antioxidant activity, with radical scavenging properties and phenolic content that may contribute to its antioxidant efficacy. It also showed cytotoxic activity against cancer cells, particularly human hepatocellular liver carcinoma (HepG2) and human colon carcinoma (HCT-116) cells, indicating their potential as a source of anti-cancer agents [6]. However, the extract exhibited moderate to weak antiviral activity and limited antimicrobial activity against specific microorganisms [6]. Antibacterial compounds, such as phenol, (Z)-9-tricosene, palmitic acid, oleamide, hexyl cinnamic aldehyde, betaine and several cinnamic aldehyde were present in all extracts of *S. cristaefolium* [7].

The volatile compositions of some *Sargassum* species have been identified. The most abundant volatile compound identified in *S. dentifolium* was phytol [6]. The volatile compounds of *S. thunbergii* were the saturated and unsaturated polyenes comprising of (6*Z*,9*Z*,12*Z*,15*Z*,18*Z*)-1,6,9,12,15,18-henicosahexaene, (6*Z*,9*Z*,12*Z*,15*Z*)-1,6,9,12,15-henicosapentaene, (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*,18*Z*)-3,6,9,12,15,18-henicosahexaene and (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-3,6,9,12,15-henicosapentaene[8]. Another sample of *S. thunbergii* from China had an abundant of pentadecane (9.24% and 13.87%), tridecanal (11.46% and 11.02%), pentadecanal (6.86% and 11.33%), 8-heptadecene (5.97% and 14.82%) and (*Z*)-11-

pentadecanal (5.49% and 5.85%)[9]. The main volatiles of mature *S. cinctum* are 1,3,5-undecatriene and 6-(1-butenyl)cyclohepta-1,4-diene, while the immature sample contained 2-ethyl-1-hexanol, 6-[(*Z*)-1-butenyl]-1,4-cycloheptadieneand 1,3,5-undecatriene [10]. The head space volatiles of *S. cinctum* exhibited insect repellency activity [10, 11].

Sargassum natans (Linn.) Gaillon is a species of brown algae in the family Sargassaceae [12]. In English the species goes by the common names such as common gulfweed, narrow leaf gulfweed, or spiny gulfweed. *S. natans* is a bushy seaweed with narrow leaf blades which are golden brown with toothed edges. The rubbery-textured leaves range from 2-6 mm (0.07-0.2 in) wide and 2-10 cm (0.8-4 in) long. The gas-filled floats are less than 6 mm (0.2 in) in diameter and are held on short stalks along the stems among the leaves. The floats of *S. natans* have a single protruding spine 2-5mm (0.07-0.2 in) long. *S. natans* does not have a single main stem; instead it grows in many directions forming clumps that can reach 60 cm (23.5 in) long. It is these clumps that form together into much larger mats [13, 14]. *S. natans* has a broad geographical distribution, being reported from coasts of America, New Zealand, southeast and southwest of coasts of Asia, Africa and Europe, including the islands of Caribbean and Atlantic Ocean [15]. *S. natans* was known to produced sodium alginate [16] and fucoidan [17] which can act as antioxidant, anticancer, antibacterial, anti-inflammatory, antiviral and hepaprotective agents [18]. The presence of flavonoids, tannins, terpenoids and saponins have been confirmed in *S. natans* extract [19], which show that the species can be harnessed for their medicinal potentials. *S. natans* was known to contained mannitol, sugar [20], heavy metals, carbohydrates and amino acids [21]. *S. natans* extract demonstrated high antibacterial activity against Gram (+) *Staphylococcus aureus* and moderate behavior against Gram (-) *Pseudomonas aeruginosa* [5, 22].

In this paper, we report the results of the phytochemical screening, chemical composition, antidiabetic and antioxidant activities of the methanol extracts of *S. natans* collected from Nigeria. In our previous communications, reports on volatile compositions and biological applications of essential oils from Nigerian flora have been published [23, 24].

2. Materials and methods

2.1 Plant material

Sargassum natans sample was collected in December 2021 at Iceland Beach, Ajah, Lagos Island, Lagos State, Nigeria. The collection was done at Latitude (6.455027^{0}N) and Longitude (3.384082^{0}E). The sample was identified by Dr. Nodza G.I. of the Herbarium, Botany Department, University of Lagos. A voucher specimen, LUH 9042 was preserved at the Herbarium.

2.2 Preparation of sample

The selected sample of *S. natans* were collected by hand, washed with seawater at the sampling site to remove impurities and sediments and then packed in polythene bags into the laboratory for further analyses. Thereafter, the sample was washed successively with distilled water to remove all the salt on the surface. The water was drained off, the clean seaweed was air-dried in the laboratory for 7 days. The dried algal material was grinded into small particle size.

2.3 Extraction of phytochemical compounds from *S. natans*

Fifty grams of the seaweed powder was soaked in 99% methanol in a separating funnel for 48 h while the funnel was subjected to shaking for 45 min and then first filtered through a double layer Muslin cloth and then filtered through Whatman filter paper. The resulting filtrate was evaporated at 64 °C via a rotary evaporator to produce a greenish syrup. The syrup paste was dried at 40 °C in an oven to get a crude extract. Until it was needed for analysis, the crude extract was kept in a refrigerator in an airtight sample bottle.

2.4 DPPH radical scavenging assay

Briefly 0.1 mM solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol was prepared. Then 1 mL of the solution was added to 1 mL of extract in water at different concentrations (25-100 µg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517 nm by using a UV-Visible Spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The percent DPPH scavenging effect was calculated using the following equation [25]

$$\text{DPPH Scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance of the extract.

2.5 α-Amylase inhibitory assay

α-Amylase inhibitory activity of extract was carried out according to the standard method with minor modification [26]. In a 96-well plate containing 50 µL phosphate buffer (100 mM, pH 6.8) was placed 10 µL of α-amylase (2U/mL) and 20 µL of varying concentrations of extract (20, 40, 60, 80 and 100 µg/mL). The mixture was pre-incubated at 37 °C for 20 min. Then, 20 µL of 1% soluble starch in 100 mM phosphate (buffer pH 6.8) was added as a substrate and incubated further at 37 °C, for 30 min. Thereafter, 100 µL of the dinitrosalicyclic acid (DNS) colour reagent was added and incubated in boiling water bath for 10 min. The solution was made to cool to room temperature. After cooling, the reaction mixture was diluted with distilled water. The absorbance of the resulting mixture was measured at 540 nm. Acarbose at several concentrations (20 µg/mL-100 µg/mL) was used as standard. The standard was set up in parallel as control and each experiment was carried out in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula.

$$\text{(\% inhibition)} = [(A_0 - A_1)/A_0] \text{ X } 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance of the extract.

2.6 α-glucosidase inhibitory assay

This study was carried out according to the standard method with minor modification [27]. To a 96-well plate containing 50 µl phosphate buffer (100 mM, pH 6.8) was added 10 µL α-glucosidase (2U/mL), and 20 µL of varying concentrations of extract (20, 40, 60, 80 and 100 µg/ml) which was pre-incubated at 37 °C for 20 min. Then, the 20 µl of 1% soluble starch in 100 mM phosphate (buffer pH 6.8) was added as a substrate and incubated further at 37 °C for 30 min. Then, 100 µL of the dinitrisalicyclic acid (DNS) colour reagent was added and incubated in boiling water bath for 10 min and then cooled to room temperature. After cooling, the reaction mixture was diluted with distilled water. The absorbance of the resulting mixture was measured at 540 nm. Acarbose at several concentrations (20 µg/mL-100 µg/mL) was used as standard. The standard was set up in parallel as control and each experiment was carried out in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula.

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance of the extract.

2.7 Gas chromatography-Mass spectrometry (GC/MS-FID) analysis

GC/MS analyses were carried out using an Agilent GC7890A system with Mass Selective Detector (Agilent 5975C). An HP-5MS fused silica capillary column (60 m ×0.25 mm i.d. × 0.25 µm film thickness) was used. Helium was the carrier gas with a flow rate of 1.0 mL/min. The inlet temperature was 250 °C and the oven temperature program was as follows: 60 °C to 240 °C at 4 °C/min. The split ratio was 100:1 and the injection volume was 1 µL. The MS analysis was carried out at interface temperature 270 °C, MS mode, E.I. detector voltage 1258 eV, and mass range 35-450 Da at 4.0 scan/s. FID analysis was carried out using the same chromatographic conditions. The FID temperature was 270 °C. The retention indices (RI) were experimentally determined using the *n*-alkanes (C_8-C_{20}) analyzed under the same GC-conditions.
The identification of volatile components was based on comparison of their retention indices, retention times and mass spectra with those obtained from authentic standards and/or mass spectral libraryof the GC-MS data system (W09N08), and the NIST Chemistry WebBook [28].

2.8 Statistical analysis

All the measurements were carried out in triplicate and the results, expressed in terms of mean ± standard deviation was calculated using Microsoft Excel software respectively.

3. Results & Discussion

3.1 Qualitative phytochemical analysis

The results of the phytochemical screening of the methanol extract of *S. natans* indicated the presence of phlobatannin, saponin, terpenoid, cardiac glycoside and alkaloids. The phytochemical quantifications showed that *S. natans* displayed a high total saponin content (38.77 mg/100g) as seen in Table 1.

Table 1: Quantification of phytochemicals from *S. natans*

Phytochemicals	Quantity (mg/100g) [a]
Saponins	38.77 ± 0.01
Alkaloids	26.55 ± 0.01
Terpenoids	9.89 ± 0.01
Cardiac glycosides	25.45 ± 0.01
Phlobatannin	21.05 ± 0.01

[a]Means of three replicates

Extracts from *S. natans* are known to contain different phytochemicals. The phytochemicals listed in Table 1, which were identified from *S. natans* from Nigeria, were previously observed in *Sargassum* samples analysed from other parts of the world [29-31].

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3.2 *In vitro* antioxidant assay

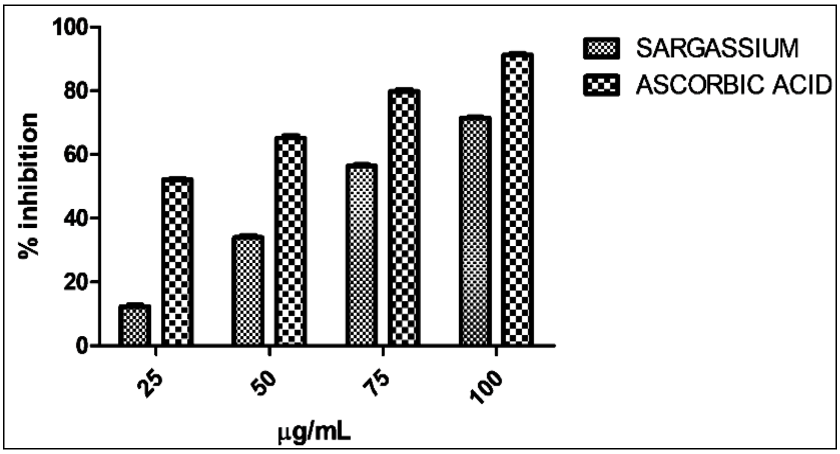

Fig 1: 2, 2-diphenyl-2-picylhydrazyl (DPPH) radicals scavenging effect of *S. natans.*

Figure 1 indicates that *S. natans* methanol extract displayed antioxidant activities with increasing concentration when compared with Ascorbic acid. The antioxidant action becomes more pronounced at the concentrations of 50, 75 and 100 µg/mL, with percentage inhibitions of 50 ± 0.01, 60 ± 0.00 and 78% ± 0.01, respectively.
The observed antioxidant potential of *S. natans* in this study is in tandem with results obtained for similar samples from Mexico [30, 32], India [29] and Jamaica [31]. Previous research showed that extracts from other *Sargassum* plants have demonstrated potent antioxidant activity in a number of models. These includes *S. fluitans*, *S. ilifolium* and *Sargassum* sp. on DPPH method [33], *S. polycystum* [34], *S. macrocarpum* [35] and *S. fluitans* [31, 32]. The crude lipid extract of tropical *S. ilicifolium* can exert antioxidant activity in DPPH model [36].

The antioxidant properties of these *Sargassum* extracts have been attributed to the presence of phenolic contents present in them [32, 33]. The antioxidant activity of *S. hystrix* was attributed to the compound fucoidan [37].

3.3 α-amylase inhibitory assay

Figure 2 shows the % inhibition of α-amylase by methanol extract of *S. natans*. The result of the assay was concentration dependent with lowest inhibition at 25 µg/mL and highest inhibition at 100 µg/mL. As the concentration increases from 25- 100 µg/mL, the percentage inhibitory potency of the methanol extract against α- amylase increases from 25% ± 0.01 to 64% ± 0.02. The standard (acarbose) displayed higher inhibitory potency at all tested concentrations.

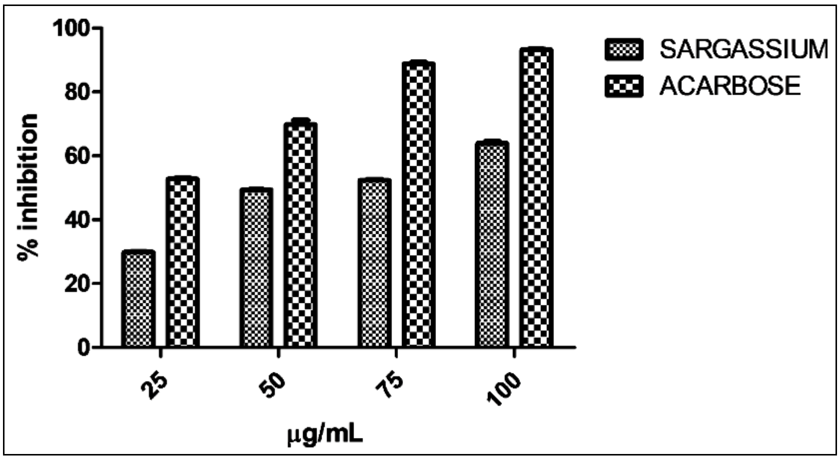

Fig 2: Inhibitory potency of *S. natans* against α-amylase activity.

3.4 α-glucosidase inhibitory assay

Figure 3 depicts the % inhibition of α-glucosidase by methanol extract of *S. natans.* The assay was concentration dependent with lowest inhibition at 25 µg/mL and highest inhibition at 100 µg/mL. At higher concentrations of 75 and 100 µg/mL, methanol extract displayed better inhibitory potency of 45% ± 0.00 and 65% ± 0.01, respectively, against α-glucosidase.

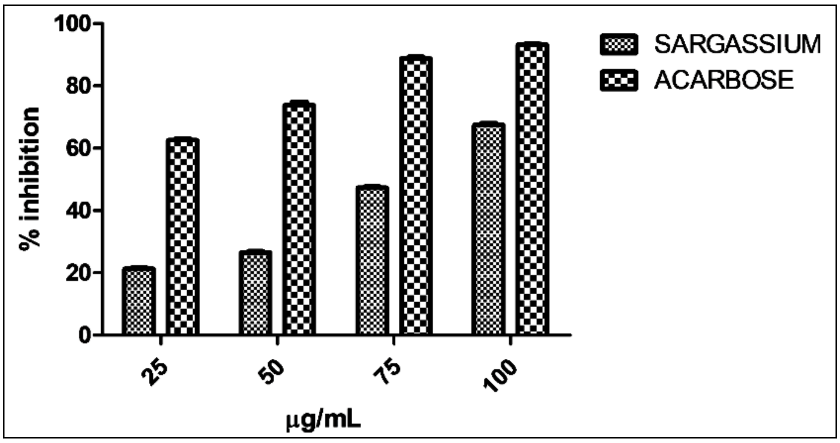

Fig 3: Inhibitory potency of *S. natans* extract against α-glucosidase activity.

The methanolic extract of *S. natnans* showed an improve anti-diabetic activities with increasing tested concentrations. This was in line with studies conducted on other S*argassum* extracts for their α-amylase and α-glucosidase inhibitory activities. In previous studies, methanolic extract of *S. glaucescens* exhibited a potent inhibition of the enzyme compared to acarbose as a positive control [38]. This result was probably due to the sterols identified in the extract. The crude extract and fucoxanthin-rich fractions of *S. siliquosum* and *S. polycystum* demonstrated α-amylase and α-glucosidase inhibitory activities [39]. *S. angustifolium* brown algae exhibited anti-diabetic activity, and has potential for lowering intestinal glucose uptake in diabetic patients [40]. Ethanol extract of *Sargassum aquifolium* suppressed the rise in postprandial hyperglycemia *in vivo* in part, through inhibition of alpha amylase and glucosidase [41]. Various extracts of *S. hemiphyllum* inhibition the enzymes of α-amylase, α-glucosidase, sucrose, and maltase activities [42].

3.5 GC/MS analysis: The compounds identified from the GC-MS analysis of the methanolic extract of *S. natans* could be seen in Table 2. There are 12 components with 2, 4-di-tert-butylphenol (58.04%) and palmitic acid (24.65%), as the dominant with higher percentages. Other notable compounds include n-nonadecanol-1 (5.98%), methyl stearate (3.22%), 2-pentadecanone (2.34%) and benzene propanoic acid (2.16%).

This is the first report on the volatile compositions of *S. natans*. The main classes of compounds are aromatic and fatty acids. This compositional pattern was different from data obtained for the volatiles of some other *Sargassum* species. For example, phytol, the most abundant volatile compound identified in *S. dentifolium* [6], unsaturated polyenes present in *S. thunbergii* [8], as well as 1,3,5-undecatriene and 6-(1-butenyl)cyclohepta-1,4-diene and 2-ethyl-1-hexanol, 6-[(*Z*)-1-butenyl]-1,4-cycloheptadieneand 1,3,5-undecatriene present in *S. cinctum* [9] were not identified in *S. natans.* Moreover, pentadecane, tridecanal, pentadecanal, 8-heptadecene and (*Z*)-11-pentadecanal of Chinese sample of *S. thunbergii* [10] could not be identified in the present *S. natans*. The differences in the compositional pattern may be attributed to factors including the difference *Sargassum* samples and the method of extraction.

Table 2: Chemical constituents of methanolic extract of *S. natans*

S/N	Compounds [a]	RI [b]	RI [c]	Percentage (%)
1	Butanoic acid	848	860	0.96 ± 0.00
2	Fumaric acid	986	971	1.47 ± 0.00
3	Benzene propanoic acid	1361	1357	2.16 ± 0.00
4	Undecanoic acid	1466	1451	0.40 ± 0.00
5	2,4-Di-tert-butylphenol	1513	1532	58.04 ± 0.02
6	2-Pentadecanone	1698	1700	2.34 ± 0.02
7	Hexadecanoic acid	1964	1966	24.65 ± 0.02
8	Methyl stearate	2085	2090	3.22 ± 0.00
9	n-Nonadecanol-1	2172	2174	5.98 ± 0.01

[a] Elution order on HP-5MS column; [b] Experimental retention indices; [c] Literature retention indices on HP-5MS column; Sr. No, serial number

The observed biological activities of *S. natans* extract may be due to the biochemical compounds identified so far. n-Hexadecanoic acid, one of the major compounds of *S. natans* has showed antioxidant potential of 30.19-89.13% at 100-500 µg/mL, in the DPPH model [43] and has contributed immensely to the anti-diabetic actions of some plant extracts [44, 45]. The antioxidant activities of the 2, 4-di-tert-butylphenol (2, 4-DTBP) has been confirmed [46]. The DPPH antioxidant assay of the 2, 4-di-tert-butylphenol (2, 4-DTBP) isolated from the brown seaweed, *Dictyota ciliolata* revealed that the higher antioxidant content of 59.02 % with the IC_{50} value of 56.61 µL when compared to the standard ascorbic acid [47]. Findings showed that the 2, 4-DTBP in bamboo shoots is a candidate compound for anti-diabetes-related enzymes [48]. The contributions of n-nonadecanol-1 to the antioxidant and anti-obesity potentials of several plant extracts have been reported [49].

4. Conclusions

This study showed that the compositional patterns of volatile compounds from the methanolic extract of *S. natans* from Nigeria, were quite different from previously reported data from other *Sargassum* sample volatilesparts of the world. In addition, the extract showed significant antioxidant, and antidiabetic potency, which increases with increasing concentrations.

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