ISSN: 2576-6732 (Print)
ISSN: 2576-6724 (Online) Acta Chemica Malaysia (ACMY) 2021, VOLUME 5, ISSUE 2

Characterization and antibacterial activities of chloroform fraction of *Laminaria saccharina*

Chinyere Benardette Chinaka Ikpa[a], Chinomnso U. Ikpa[b], Gladys C. Ezeh[c], Samuel Okwudili Onoja[d]*

[a] Department of Chemistry, Imo State University P.M.B 2000, Owerri, Imo State Nigeria.
[b] Department of Chemistry/Biochemistry/Molecular Biology, Alex Ekwueme Federal University of Agriculture, Ndufu Alike-Ikwo, Eboinyi State, Nigeria.
[c] Department of Agriculture, Alex Ekwueme Federal University of Agriculture, Ndufu Alike-Ikwo, Eboinyi State, Nigeria.
[d] Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture Umudike, PMB 7267, Umuahia Abia State Nigeria.
*Corresponding Author Email: onoja.samuel@mouau.edu.ng

DOI: 10.2478/acmy-2021-0011

Abstract:
Laminaria saccharina is an aquatic plant mainly used as dietary fibre in Europe, America and Japan. This study investigated the phytochemical composition and antibacterial properties of the chloroform extract of *Laminaria saccharina* leaves. The phytochemical composition and antibacterial properties of the leaves were evaluated using standard methods. The chloroform fraction of the leaves extract was used for antibacterial screening. The chloroform fraction was fractionated and the pure compound isolated was characterized using infrared (IR), nuclear magnetic resonance (NMR) and mass spectrometer. The phytochemical result showed the presence of alkaloid 2.5 ± 0.20%, phenol 0.38 ± 0.22%, flavonoids 3.60 ± 0.20%, saponins 1.00 ± 0.20% and tannins 0.05 ± 0.20% w/w. The functional groups present in IR absorption bands, the signals on the NMR and mass of 477 m/z on mass spectrometer confirmed the characterization and elucidation of a glycosaccharide (compound 1) called 12-ethyl-5,8-dihydroxy-7-(hydroxymethyl) cyclopentaquinoline-2-one-6-methoxyglucopyronose with calculated mass of 473.514 m/z. The extract inhibited the growth of both Gram-positive and Gram-negative bacterial isolates. The minimum inhibitory concentration (MIC) of the extract against *Staphylococcus aureus*, *Escherichia coli, a*nd *Proteus mirabilis* was 1.25 mg/ml and 2.50 mg/ml against *Streptococcus pneumonia*. The chloroform fraction of *L. saccharina* inhibited the growth of both Gram-positive and Gram-negative organism and could be used in the treatment of bacterial infection as a broad-spectrum antibacterial agent.

Keywords: Aquatic plants; Bioactive compound; *Laminaria saccharina*; Minimum inhibitory concentration; Phytoconstituent

1.0. Introduction

The resistance of pathogenic organisms against antimicrobial agents is a challenge to public health worldwide [1]. About 700,000 death are recorded worldwide, annually due to antimicrobial resistance [2]. The annual death rate attributed to antimicrobial resistance is projected to be 10 million by 2050 [3]. To reverse this trend, the World Health Organisation has recommended the production of new antimicrobial agent. One of the commonly exploited sources of new drugs are medicinal plants. These medicinal plants are selected based on their folkloric uses. Marine plants with medicinal values are used in the coastal and riverine areas for health-care need of the populace. A lot of novel structural and pharmacologically important compounds with antimicrobial, antitumor and anti-inflammatory properties have been isolated from aquatic plants [4]. Marine organisms have long been recognized as a source of novel metabolites with applications in human disease therapy [5]. The use of aquatic plants in herbal medicine system is gaining ground but the chemical composition has been under studied [6]. Some of the commonly studied marine plants are *Laminaria digitata*, *Laminaria saccharina* and *Himanthalia elongata* (*Phaeophyta*) [7].

Laminaria saccharina, known as "*Saccharina latissima* or Sugar kelp" in Europe, America and Japan [8] and "Sugar kombu" in Spain [9]. It belongs to the family *Laminariaceae* and is found in Atlantic and Pacific Ocean and other water bodies in the tropics [10]. It has a yellowish-brown colour with long narrow unbranched blade; that may be about 5 metre in length and 20 centimetre in width. It has a smooth wavy edge and the frond is anchored to rock by strong rhizoid of about 5 milimetre diameter. *Laminaria* is commonly used in soup, candy, and sushi, or is eaten with rice as a salad [10]. The cytotoxic, immunomodulatory, and antioxidant activities of *L. saccharina* have been reported [7, 11, 12]. The leaves of *L. saccharina* is used in traditional management of bacterial infection, but there is dearth of information on the scientific evaluation of the acclaimed antibacterial properties. In the face of the challenges of antibacterial resistance, this study was designed to isolate and characterize a novel antibacterial agent from chloroform fraction of *Laminaria saccharina* leaf.

2.0. Experimental

2.1. Plant materials

Matured leaves sample of seaweed *Laminaria saccharina* were harvested in Aba River (water side) in Obingwa Local Govement Area (5°2'30''N to 5°10'00" and 7°20'0'' to 7°25'0''E), Abia State. The plant was authenticated by Prof. M. C. Dike of Department of Forestry, Michael Okpara University of Agriculture Umudike Nigeria. The leaves were dried at room temperature under natural lighting for 28 days. The leaves were ground to powder and stored in an amber bottle. Aliquot of the powdered leaves sample was used for phytochemical analysis. The rest of material was percolated with 2 L of ethanol (90% analytical grade) at room temperature for four days. The extract was filtered and concentrated to dryness with rotary evaporator for 8 hours at 40 °C.

2.2. Phytochemical analysis

The *L. saccharina* leaf was evaluated for content of alkaloids and phenols according to the methods described by Harborne [13]. The flavonoids content was evaluated as described by Boham and Kocipai [14]. The tannins and saponins contents were determined as reported by Obadoni and Ochuko [15]. All tests were done in triplicate.

2.3. Fractional separation of the compounds

The partitioning of the ethanol extract with different solvents to obtain various fractions was done as described by Ikpa *et al* [16]. 10 g of the dry extract was partitioned between chloroform and water to obtain chloroform and water fractions. The chloroform fraction of *Laminaria saccharina* (CFLS) was subjected to column chromatography over silica gel (Merck grade, 60-120 mesh). The eluted fractions were analysed with TLC and the fraction that gave one spot with Rf value of 0.58 was further analysed [16]. The pure compound was characterized using FT-IR, mass was determined with TOF MS positive ion mode while ^{1}H and ^{13}C NMR was determined at University of Sheffield using AV 400 model.

2.4. Evaluation of antibacterial activity of CFLS

2.4.1. Preparation of the inoculums

The standard clinical isolates of Gram-positive (*Staphylococcus aureus*, *Streptococcus pneumonia*) and Gram-negative (*Escherichia coli a*nd *Proteus mirabilis*) organisms were obtained from Microbiology Unit of Federal Medical Centre, Owerri. The antibacterial analysis was carried out at the Department of Medical Science Laboratory, Imo State University. These organisms were propagated on nutrient agar plates and maintained at 40 ^{o}C. The isolates were sub-cultured in nutrient broth at 37 ^{o}C for 24 h prior to antibacterial assay.

2.4.2. Antibacterial sensitivity test of CFLS

Antibacterial activity of the CFLS against the test microorganisms were determined by Agar well diffusion technique as described by Paliwal *et al* [17]. Sterile agar plates were inoculated with 0.1 ml of overnight culture of each bacteria strain (equivalent to 108 CFU/ml). A plastic cork-borer (6 mm in diameter) was used to create wells on the inoculated nutrient agar. 300 µl of different concentrations of CFLS (0.3125, 0.625, 1.25, 2.50, 5.00 and 10.00 mg/ml) dissolved in dimethyl sulphoxide (DMSO) were delivered into the wells in triplicate. The plates were left on the bench for 30 minutes to allow the compound to diffuse into the agar. Thereafter, the plates were incubated at 37 ^{o}C for 24 hours. After incubation, the plates were observed for zones of inhibition around the wells and the diameters of the zones were measured with metre rule.

2.5. Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA). Least Significant Difference (LSD) was used to separate mean difference and significance was accepted at the level of $p < 0.05$.

3.0. Results

3.1. Phytochemical analysis of L. saccharina leaves

Analysis by (method) showed that the alkaloids and flavonoids composition in seaweed were 2.51% and 3.60% w/w, respectively. Further, the contents of the phenols, saponins and tannins compositions were 0.35%, 1.00% and 0.05% w/w, respectively (Table 1).

Table 1. Phytochemical analysis of *L. saccharina* leaves

Phytochemical	Content (%)
Alkaliods	2.51 ± 0.20
Phenols	0.35 ± 0.22
Flavoniods	3.60 ± 0.20
Saponins	1.00 ± 0.20
Tannins	0.05 ± 0.02

Value=SD of triplicate values

3.2. Antibacterial sensitivity test of CFLS

The CFLS was tested for antibacterial activity against selected bacteria. The results showed concentration dependent increase in the zone of inhibition against the test organisms. The minimum inhibitory concentration (MIC) of the CFLS against *S. aureus, E. coli* and *P. mirabilis* was 1.25 mg/ml, while the MIC against *S. pneumonia* was 2.5 mg/ml (Table 2).

Table 2. Zone of inhibition of CFLS

Organisms	Zone of inhibition (mm)						
	0.31 mg/ml	0.63 mg/ml	1.25 mg/ml	2.50 mg/ml	5.00 mg/ml	10.00 mg/ml	MIC (mg/ml)
S. aureus	0	0	2.00 ± 0.15	4.00 ± 0.21	10.07 ± 0.03	16.00 ± 0.10	1.25
S. pneumonia	0	0	0	1.03 ± 0.09	7.97 ± 0.09	17.03 ± 0.03	2.50
E. coli	0	0	2.03 ± 0.03	5.07 ± 0.07	13.13 ± 0.13	18.10 ± 0.06	1.25
P. mirabilis	0	0	1.03 ± 0.03	5.07 ± 0.07	10.07 ± 0.07	18.10 ± 0.10	1.25

3.3. Characterization of isolated pure compound

The column chromatography gave light green oil with Rf of 0.58 on TLC. The determined mass of the pure compound by TOF Mass Spectroscopy was 477 m/z while, the calculated mass was 473.514 m/z. The IR spectrum exhibited absorptions (in cm^{-1}) at 2852.2 (R-H saturated), 1739.6 (saturated lactone). 1463.0 (-OH bending) 1376.4 (2^{o} amine) and 1097.3 (C-OH stretching) (Table 3). NMR analysis of the isolated compound (**1**) is shown in Table 4. The ^{1}H NMR spectrum of compound (**1**) showed CH_2 proton at δH: ($0.70_{(t)}$, $0.80_{(t)}$, $1.00_{(t)}$ and $1.60_{(d)}$), CH proton at δH: ($2.00_{(d)}$ and $2.40_{(m)}$), CH proton with -0H at δH: $2.90_{(s)}$ with coupling constant of J= 1.14, –OH protons at δH: 6.30 and NH proton at δH: $7.20_{(s)}$ while the protons of sugar moity were indicated at δH: 320-540 with J-value of 25.24 ^{13}C NMR showed signals of aliphatic methylene carbons at δC: (16.00 and 22.71), while the fused alicyclic methylene carbons (-CH_2-) were indicated at δC: (27.22 & 29.71), methine carbons (-CH-) at δC: (31.94, 76.71 &77.03), quaternary carbons at δC: 77.35 while the keto carbon (C=O) was indicated at δC: 107.00. The absorbance of the FT-IR and the signals of both proton and carbon NMR were combined to elucidate the structure of a glycosaccharide called 12-ethyl-5,8-dihydroxy-7-(hydroxymethyl) cyclopentaquinoline-2-one-6-methoxyglucopyranose.

Table 3. Results of FTIR Spectroscopy of the isolated compound **1**

PEAKS in cm-1	FUNCTIONAL GROUPS	REMARKS
2852.2	R-H	Saturated alkane
1739.6	C=O	Saturated lactone
1463.0	-OH	Bending
1376.4	-NH-	Secondary amine
1097.3	C-OH	Stretching

Table 4. Results of NMR Spectroscopy of Compound 1

Assign	Fragment	Int. Value	δ ^{1}H	J. value	δ ^{13}C:	Multiplicity
1	NH	1	7.20			-
2	-C-	-			107.00	-
3	CH_2	2	0.70		29.71	m
4	CH_2	2	1.00		29.71	-
5	-C-	-			77.35	-
6	CH	1	2.40		76.71	-
7	CH	1	2.00		31.94	d
8	-C-	-			77.35	-
9	-C-	-			77.03	-
10	CH	1	1.60		31.94	q
11	CH_2	2	0.70		27.22	m
12	CH	1	2.00		31.94	m
13	CH_2	2	0.80		27.22	q
14	CH_2	2	0.70		16.00	m
15	CH_2	2	1.00		16.00	m
16	CH_2	2	2.90	1.14	22.71	q
	-OH	1x3	6.30		-	-
Sugar	$C_7H_{13}O_6$	13	3.20-5.40	25.25	-	-

d = dublet, q = quatet, m = multiplet, Int. value = Integral value

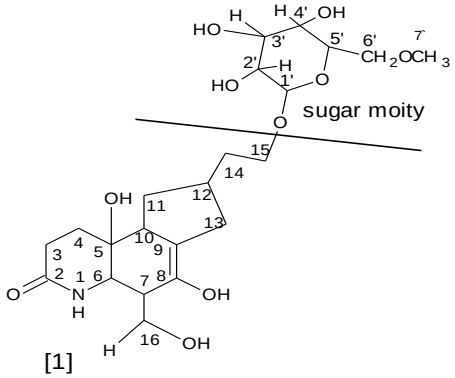
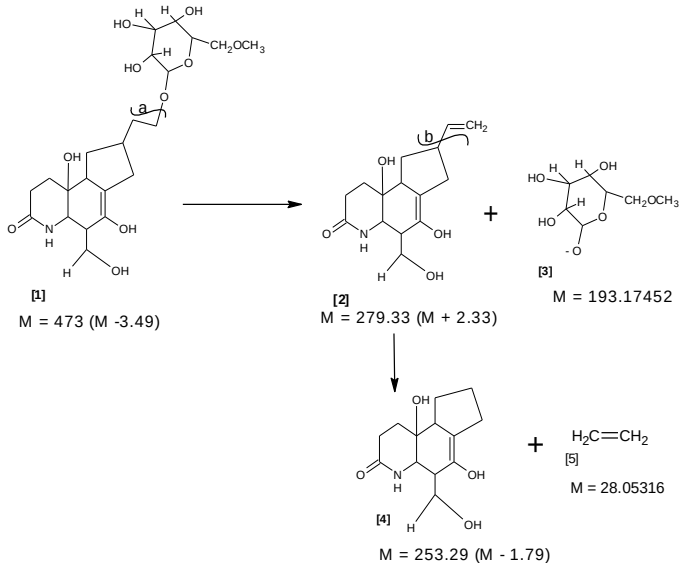


Figure 1. Structure of the isolated compound **(1)**, named 12-ethyl-5,8-dihydroxy-7-(hydroxymethyl) cyclopentaquinoline-2-one-6-methoxyglucopyranose

The fragmentation resulted in compounds **(2-5)**. The isolated compound contains a sugar molecule with mass of 193.17452 and formula of $C_7H_{13}O_6$ called methoxyglucopyranose **(3)** and an aglycone called 8-ethnyl-5,8-dihydroxy-7-(hydroxylmethyl) decahydrocyclopentaquinoline-2-one **(2)**. Further fragmentation of compound **(2)** gave compound **(4)** called 5,8-dihydroxy-7-(hydroxymethyl) cyclopentaquinoline-2-one and ethene **(5)** (C_2H_4). The relationship of theoretical masses to corresponding experimental masses were used to confirm the characterization of the compound (Scheme 1).



Scheme 1. Fragmentation of the elucidated compound

Legend: (**1**) = 12-ethyl-5,8-dihydroxy-7-(hydroxymethyl) cyclopentaquinoline-2-one-6-methoxyglucopyranose, (**2**) = 8-ethnyl-5,8-dihydroxy-7-(hydroxylmethyl) decahydrocyclopentaquinoline-2-one, (**3**) = methoxyglucopyranose, (**4**) = 5,8-dihydroxy-7-(hydroxymethyl) cyclopentaquinoline-2-one, (**5**) = ethene

4.0. Discussion

The chloroform fraction of *L. saccharina* (CFLS) leaves elicited potent antibacterial activity against *S. aureus, S. pneumonia, E. coli* and *P. mirabilis* which indicates potential use against both Gram-positive and Gram-negative organisms. The antibacterial activity of CFLS could be linked to its phytoconstituents (alkaloids, flavonoids, tannins, saponins and phenols) and most especially the isolated glycosaccharide; 12-ethyl-5,8-dihydroxy-7-(hydroxymethyl) cyclopentaquinoline-2-one-6-methoxyglucopyranose. The presence of these phytochemicals in *L. saccharina* have been reported by other investigators and the difference in the content could be attributed to the environmental conditions of where it was harvested and the solvent used in extraction [12]. Sharma *et al* [18] reported that the growth and phytochemical composition of algae are influenced by environmental factors such as the salinity of seawater, pH, sunlight, mineral availability, waves, and water current. The antibacterial activities of the phytoconstituents of the *L. saccharina* have been reported and it is in agreement with the findings of previous researchers [10, 19]. The antibacterial activities of flavonoids and phenols isolated from medicinal plants have been documented [20]. Tannins and saponins are known to be toxic to bacteria [21, 22]. The antibacterial property of phlorotannins, a polyphenol isolated from seaweeds, has been reported and its mechanism of actions are via the inhibition of oxidative phosphorylation enzymes and cell lysis [23].

The concentration-dependent increase in the zone of inhibition of the bacterial isolate corroborated the reports of Gupta and co-workers [10]. The MIC of the CFLS against the test organisms ranged between 1.25 – 2.5 mg/ml. This indicates that the CFLS have potent antibacterial activity. The isolated glycosaccharide; 12-ethyl-5,8-dihydroxy-7-(hydroxymethyl) cyclopentaquinoline -2-one-6-methoxyglucopyranose (1) have cyclopentane ring, keto, hydroxyl and heterocyclic nitrogen functionalities which have been associated with antibacterial properties [24, 25]. The mechanism of the antibacterial activity was not evaluated and thus not known. The antibacterial activity could be via interaction with the glycoprotein receptors on the bacterial cell wall, cell membrane and deoxyribonucleic acid (DNA). Antibacterial activities of two polysaccharides, fucoidan and laminarin isolated from seaweeds, against *S. aureus, E. coli* and *Helicobacter pylori* have been reported [23]. These polysaccharides elicit their antibacterial effects via the interaction with glycoprotein receptor leading to impaired cytoplasmic membrane permeability, protein leakage and DNA damage [26].

5.0. Conclusion

This study validated the use of *Laminaria saccharina* leaf in the traditional treatment of bacterial diseases by the people of South-Eastern Nigeria. The antibacterial activity of *L. saccharina* could be linked to the presence of glycosaccharide; 12-ethyl-5,8-dihydroxy-7-(hydroxylmethyl)cyclopentaquinoline-2-one-6-methoxyglucopyranose **(1)** and other phytoconstituents. The isolated compound (1) can be used as a starting material in the production of antibacterial agent that could be used in the treatment of bacterial infection.

6.0. Acknowledgement

The authors are grateful to Prof. M. C. Dike for the identification of the plant sample.

7.0. References

[1] A. Jesline, N.P. John, P.M. Narayanan, C. Vani, S. Murugan, "Antimicrobial activity of zinc and titanium dioxide nanoparticles against biofilm-producing methicillin-resistant *Staphylococcus aureus", Appl. Nanosci.,* Vol. 5, No. 2, Pp. 157-62, 2015.

[2] P. Srivastava, M. Shukla, G. Kaul, S. Chopra, and A. K. Patra, "Rationally designed curcumin-based ruthenium (II) antimicrobials effective against drug-resistant *Staphylococcus aureus"*, Dalton Trans., Vol. 48, No. 31, Pp. 11822-11828, 2019.

[3] H. Sanderson, R.S. Brown, P. Hania, T.A. McAllister, A. Majury, and S.N. Liss, "Antimicrobial Resistant Genes and Organisms as Environmental Contaminants of Emerging Concern: Addressing Global Public Health Risks", In Management of Emerging Public Health Issues and Risks, Academic Press, United States, Pp. 147-187, 2019.

[4] P. Bhadury, and P.C. Wright, "Exploitation of marine algae: Biogenic compounds for potential antifouling applications", Planta., No. 219, Pp. 561-578, 2004.

[5] S. Vignesh, A. Raja, and R. A. James, "Marine drugs: Implication and future studies", Int. J. Pharmacol., Vol. 7, No. 1, Pp. 22-30, 2011.

[6] M. Aasim, K.M. Khawar, S.I. Ahmed, and M. Karataş, "Multiple Uses of Some Important Aquatic and Semiaquatic Medicinal Plants", In: M. Ozturk, K. Hakeem (Eds.), Plant and Human Health, Volume 2. Springer, Cham, Pp. 541-577, 2019.

[7] G. Rajauria, "In-Vitro Antioxidant Properties of Lipophilic Antioxidant Compounds from 3 Brown Seaweed", Antioxidants, Vol. 8, No. 12, Pp. 596, 2019.

[8] A. Jimenez-Escrig, and I.C. Goñi, "Nutritional evaluation and physiological effects of edible seaweeds", Arch. Latinoam. Nutr., 49 (2), Pp. 114-120, 1999.

[9] P. Ruperez, and F. Saura-Calixto, "Dietary fibre and physicochemical properties of edible Spanish seaweeds", Eur. Food Res. Technol., Vol. 212, No. 3, Pp. 349-354, 2001.

[10] S. Gupta, G. Rajauria, and N. Abu-Ghannam, "Study of the microbial diversity and antimicrobial properties of Irish edible brown seaweeds", Int. J. Food Sci. Tech., Vol. 45, No. 3, Pp. 482–489, 2010.

[11] P.K. Sappati, B. Nayak, G.P. VanWalsum, and O.T. Mulrey, "Combined effects of seasonal variation and drying methods on the physicochemical properties and antioxidant activity of sugar kelp (*Saccharina latissima*)", J. Appl. Phycol., Vol. 31, No. 2, Pp. 1311-1332, 2019.

[12] M.M. Stefaniak, M. Gudjónsdóttir, G. Marteinsdottir, S. Omarsdottir, E. Bravo, O.E. Sigurjónsson, and K. Kristbergsson, "Determination of bioactive properties of food grade extracts from Icelandic edible brown seaweed sugar kelp (*Saccharina latissima*) with *in vitro* human cell cultures (THP-1)", Funct. Food Health Dis., Vol. 9, No. 1, Pp. 1-5, 2019.

[13] J.B. Harborne, "Phytochemical Methods: A guide to Modern Techniques of Plant Analysis Chapman and Hall Int", New York, Pp. 488-493, 1998.

[14] A.B. Boham, and A.C. Kocipai, "Flavonoid and Condensed Tannins from Leaves of *Hawaiian vaccininum* and *Vicalycinium vaticulum"*, Pac. Sci., Vol. 48, Pp. 458-463, 1994.

[15] B.O. Obadoni, and P.O. Ochuko, "Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria", Global J. Pure Appl. Sci., Vol. 8, No. 2, Pp. 203-208, 2002.

[16] C.B. Ikpa, F.C. Ibe, and C.U. Ikpa, "Isolation, chemical composition, characterization and anti-bacterial activity of acridine diglycoside from *Moringa olifera"*, Int. J. Pharmacol. Phytochem. Ethnomed., Vol. 2, Pp. 30-36, 2016.

[17] S.K. Paliwal, B. Sati, S. Faujdar, and S. Sharma, "Antioxidant and antibacterial activities of various extracts of *Inula cuspidata* CB Clarke stem", Beni-Seuf Univ. J. Appl. Sci., Vol. 6, No. 2, Pp. 97-105, 2017.

[18] S. Sharma, L. Neves, J. Funderud, L.T. Mydland, M. Øverland, and S.J. Horn, "Seasonal and depth variations in the chemical composition of cultivated *Saccharina latissimi"*, Algal Res., Vol. 32, Pp. 107-112, 2018.

[19] S. Cox, N. Abu-Ghannam, and S. Gupta, "An assessment of the antioxidant and antimicrobial activity of six species of edible Irish Seaweeds", Int. Food Res. J., Vol. 17, Pp. 205–220, 2009.

[20] W.Y. Huang, Y.Z. Cai, and Y. Zhang, "Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention", Nutr. Cancer., Vol. 62, No. 1, Pp. 1-20, 2009.

[21] A. Banso, and S.O. Adeyemo, "Evaluation of antibacterial properties of tannins isolated from *Dichrostachys cinerea",* Afr. J. Biotechnol., Vol. 6, No. 15, Pp. 1785-1787, 2007.

[22] L. Vallette, C. Rabadeaux, J. Sirdaarta, C. Davis, and I.E. Cock, "An upscaled extraction protocol for *Tasmannia lanceolata* (Poir.) AC Sm.: Anti-bacterial", anti-Giardial and anticancer activity, Pharmacogn. Commun., Vol. 6, No. 4, Pp. 238-254, 2016.

[23] E. Shannon, and N. Abu-Ghannam, "Antibacterial derivatives of marine algae: An overview of pharmacological mechanisms and applications", Mar. Drugs., Vol. 14, No. 4, Pp. 81, 2016.

[24] M.T. El-Sayed, S. Suzen, N. Altanlar, K. Ohlsen, and A. Hilgeroth, "Discovery of bisindolyl-substituted cycloalkane-anellated indoles as novel class of antibacterial agents against *S. aureus* and MRSA", Bioorg. Med. Chem. Lett., Vol. 26, No. 1, Pp. 218-221, 2016.

[25] M.S. Nair, D. Arish, and R.S. Joseyphus, "Synthesis, characterization, antifungal, antibacterial and DNA cleavage studies of some heterocyclic Schiff base metal complexes", J. Saudi Chem. Soc., Vol. 16, No. 1, Pp. 83-88, 2012.

[26] N.N. Besednova, T.S. Zaporozhets, L.M. Somova, and T.A. Kuznetsova, "Prospects for the use of extracts and polysaccharides from marine algae to prevent and treat the diseases caused by *Helicobacter pylori*", Helicobacter, 2015, 20(2), 89-97, 2015.