ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

EVALUATION OF RED SEAWEED *AHNFELTIA PLICATA* (HUDSON) FRIES FROM ALIBAUG COAST FOR ITS CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY

Ravinder Sah*, Sunil Shankhadarwar

Department of Botany, Ramnarain Ruia Autonomous College, Matunga (E), Mumbai, Maharashtra, India

**Corresponding author: ravindersah05@gmail.com*

ABSTRACT

The present study intends to evaluate the chemical composition, antioxidant activity and GC-MS analysis of marine red seaweed *Ahnfeltia plicata*. The Alga was collected, shade dried, ground into a fine powder and extracted with eight different solvents to examine their extractive value and phytochemical constituents. The chemical components present in *Ahnfeltia plicata* were analyzed, and its free radical scavenging activity was determined by the DPPH assay. The seaweed was subjected to GC-MS analysis which revealed the presence of 20 different compounds. From the present research, it is concluded that *Ahnfeltia plicata* is a rich source of bioactive molecules.

Keywords: Phytochemical Analysis, Biochemical, GC-MS, Antioxidant, *Ahnfeltia plicata*

1. INTRODUCTION

Marine seaweeds are an abundant source of naturally occurring diverse bioactive compounds since ancient times. Based on the habitats, maturity and environmental conditions their chemical composition varies from species to species [1]. Seaweeds are rich in protein, carbohydrate, and polyunsaturated fatty acids, vitamins and minerals that are an aid to human health [2]. Seaweeds are known to possess enormous biologically important a component like carotenoids, fucoidans, phlorotannins, flavonoids, Fucoxanthin [3] and algal polyphenol which shows various biological activities like antibacterial, antiviral, anti-inflammatory, antifouling, cytotoxic and antimitotic [4]. Due to high phenolic content, marine seaweeds are the richest source of natural antioxidants and their properties can be utilized in the prevention and treatment of neurodegenerative diseases caused by oxidative stress and cancer [5, 6].

Ahnfeltia plicata (Hudson) E.M. fries belonging to Ahnfeltiaceae family of Rhodophyta is considered as one of the most important commercially agarophytes [7] producing a high-grade agar in which sulfate content is quite low [8]. Some species of *Ahnfeltia*, are recognized as an important source of phycocolloids [9].

Hence, the present study was carried out to analyze the phytochemicals, biochemical, antioxidant activity and GC-MS analysis of *Ahnfeltia plicata*, to identify their active chemical composition.

2. MATERIAL AND METHODS

2.1. Collection and Authentication of Seaweed

Ahnfeltia plicata was collected from coastal areas of Ailbaug, Raigad district, Maharashtra India, during the low tides and authenticated by Botanical Survey of India, Coimbatore- 641003, Tamil Nadu.

2.2. Preparation of extract

Ten gram of seaweed powder is cold macerated with 100 ml of different solvents, *i.e.* Acetone, Methanol, Ethanol, Chloroform, Petroleum Ether, Ethyl acetate, distilled water for 24 hours, six hours on a shaker at 120 rpm and 18 hours standing. The extract was filtered by using whatman filter paper and evaporated on a water bath, then reconstituted by using a suitable solvent and making the final volume to 100 ml. The extract was stored in the refrigerator for further evaluation. The residue left after a filter is dried until constant weight and used for calculating extractive value.

2.3. Qualitative Phytochemical Screening

Qualitative analysis of alkaloids, carbohydrates, saponins, glycosides, protein, amino acid, phytosterol, phenol, flavonoids, Terpenoids, tannins, coumarins, of acetone, methanol, ethanol, petroleum ether, ethyl acetate, chloroform, n-hexane and aqueous seaweed extract was carried out by using a standard protocol [10,11] to identify the presence of active constituents.

2.4. QUANTITATIVE ANALYSIS

2.4.1. Determination of Phenol

The phenol content of the extract was estimated by the standard method [12]. By using a spectrophotometer the absorbance of the extract was measured at 765 nm. The quantification of phenol (mg/g) was carried out by using a standard graph of Gallic acid.

2.4.2. Estimation of Flavonoids

The estimation of Flavonoids was carried out by a standard protocol [13]. At 510 nm the absorbance of the sample mixture was recorded by using a spectrophotometer. The amount of Flavonoids contents (mg/g) was calculated by using a standard graph of Quercetin.

2.4.3. Quantification of Tannins

The Amount of tannins present in the extract was estimated as described [12]. The absorbance of the reaction solution was noted at 700 nm by using a spectrophotometer. The total amount of tannin content was calculated by using the standard graph of Tannic acid.

2.5. Biochemical Analysis

2.5.1. Estimation of Protein

The protein estimation was done by Lowry's method [14]. At 620 nm absorbance of the resultant solution was recorded by using a spectrophotometer and the total protein (mg/g) content of seaweed was estimated by a standard graph of bovine albumin serum.

2.5.2. Estimation of Total Soluble Sugar

Estimation of total soluble sugar was done by the Anthrone method. 0.1g of seaweed powder was weighed and hydrolyzed with 5ml HCl (2.5N) in a boiling water bath for 3hrs. The solution is cooled at room temperature and neutralized with sodium carbonate. Then the final volume is makeup up to 100ml by using distilled H2O and centrifuged. The supernatant was collected and used for total soluble sugar analysis. 1ml of aliquots were mixed with 4ml of Anthrone reagent and heated in boiling water bath for 8 minutes. The absorbance was recorded of green color developed after cooling at 630 nm. Glucose was used as a standard and the amount of total soluble sugar is expressed in mg/gm.

2.5.3. Estimation of Lipid

The lipid estimation was done by the Folch method [15].

2.6. Estimation of Pigments

2.6.1. Extraction

One g of seaweed is crushed with 20 ml 80% acetone in motor and pestle. The homogenate was centrifuged for 15 minutes at 3000 rpm and the supernatant was stored separately. The pellet was re-extracted until it becomes colorless by using 80% acetone. All supernatants were pooled together and used for pigments quantification.

2.6.2. Estimation of Chlorophyll

The chlorophyll content of seaweed was estimated by Arnon method [16]. By using UV-VIS Spectrophotometer the absorbance was measured at 645 nm and 663 nm. The amount of chlorophyll present in (μg/ml) was calculated by using standard Arnon's equations:

Chlorophyll a = 12.7 (A663) - 2.69 (A645)
Chlorophyll b = 22.9 (A645) - 4.68 (A663)
Total chlorophyll = 20.2 (A645) + 8.02 (A663)
Where, A = Absorbance at a corresponding wavelength.

2.6.3. Estimation of Carotenoids

The amount of Carotenoids present in seaweed was estimated by Kirk and Allen method [17]. Carotenoids content (μg/g.fr.wt.) of the same chlorophyll extract was measured at 480 nm in the UV-VIS spectrophotometer.

Carotenoids = A480 + (0.114 × A663) — (0.638 × A 645) Where, A = Absorbance at respective wave length.

2.6.4. Estimation of Fucoxanthin

The amount of fucoxanthin present was estimated as described [18]. The fucoxanthin content (mg g-1) of same chlorophyll the extract was measured at 470 nm, 631 nm, 581 nm, 664 nm, in UV-VIS spectrophotometer. Fucoxanthin = [A470 −1.239 (A631+A581−0.3 ×A664) − 0.0275(A664)] /141 Where, A = Absorbance at particular wavelength.

2.7. Estimation of Phycobillins

Five hundred mg of dried powdered was crushed in 5ml of distilled water and centrifuged at 5000 rpm for 10 min, the pellet washed with water until loss of pigment. The pellet is suspended in 3ml PO_4 buffer and homogenize, the content was freeze and thaw repeatedly and centrifuge for 5 min at 5000 rpm to ensure complete extraction. The absorbance of the collected supernatant was measured at 565nm, 615nm and 625 nm, respectively against PO_4 buffer blank. The amount of C-

phycocyanin (PC), AlloPhycocyanin (APC) and C-Phycoerythrin (PE) are calculated by using the following formulas.

$$\text{C-phycocyanin (PC)} = \frac{A615-0.474\,(A652)}{5.34}\ \mu g\ ml^{-1}$$

$$\text{Allophycocyanin (PC)} = \frac{A652-0.208\,(A615)}{5.09}\ \mu g\ ml^{-1}$$

$$\text{C-phycocyanin (PC)} = \frac{A562-2.41\,(PC)-0.849(APC)}{9.62}\ \mu g\ ml^{-1}$$

2.8. Antioxidant activity

Free radical scavenging activity was carried out by the DPPH method, 1ml of DPPH (0.004%) was added to 10ml to 1000ml of plant extract and incubated in dark at room temperature for 30 min. the absorbance of the solution was noted at 517 nm by using a spectrophotometer. The antioxidant activity of an extract was expressed in IC_{50} (mg/ml) and calculated by using the following formula.

% inhibition = (Absorbance of control-Absorbance of test/ Absorbance of control) x100

2.9. GC-MS Analysis of Seaweeds

Ethanolic extract of seaweed was analyzed in the Shimadzu GCMS-QP 2010 Ultra system equipped with Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) capillary column of 30m length, 0.25 µm internal diameter and 0.25 µm film thickness. Helium was used as carrier gases. The ionization energy of 70 eV was used for the electron ionization system.

For an ethanolic extract of *Ahhfeltia plicata*, initial oven the temperature was programmed at 40°C for 2 min and then it was raised to 250°C at the rate of 4°C/ min and held for 10 min. Finally, the oven temperature was increased to 250°C at the rate of 7°C/min. The Ion source temperature and injection temperature was 200°C and the interface temperature was set at 250°C.

3. RESULTS AND DISCUSSION

3.1. Extractive Value

The extractive value of the crude powder of *Ahnfeltia plicata* is presented in Table 1. The maximum soluble extractive value 10% was found in Methanol, while the minimum soluble extractive value 0.5% was found in n-hexane. The water-soluble extractive value was 23.20%. A similar finding was reported on *Sargassum wightii* and *Padina gymnospora* [19]. *Caulerpa racemosa* also showed maximum methanolic and the water-soluble extractive value [20].

Table 1: Percentage extractive value of different solvents

Solvents	Extractive value
Acetone	1.40%
Methanol	10%
Ethanol	8.40%
Petroleum Ether	1%
n-hexane	0.5%
Ethyl acetate	0.86%
Chloroform	0.66%
Water	23.20%

Table 2: Phytochemical compound detected in different extracts of *Ahnfeltia plicata*

Phytochemicals	Acetone	Methanol	Ethanol	Petroleum ether	Ethyl acetate	Chloroform	n-hexane	Aqueous
Alkaloids	+	+	+	-	-	-	-	-
Saponins	-	-	+	-	-	-	-	+
Glycosides	-	-	-	-	-	-	-	-
Amino acids	+	+	+	-	-	-	-	-
Phytosterol	+	-	+	-	+	+	-	-
Phenols	+	+	+	-	-	-	-	-
Flavonoids	+	+	+	-	+	+	-	+
Terpenoids	-	+	-	-	-	-	-	-
Tannins	+	+	+	-	-	-	-	+
Coumarins	+	+	+	-	+	+	-	+

+: Present, - : Absent

3.2. Phytochemicals Analysis

Phytochemical screening is essential to identify different phytochemicals compound present. In present research, acetone, methanol, ethanol, petroleum ether, ethyl acetate, chloroform, n-hexane, and aqueous extracts of *Ahnfeltia plicata* were used for the analysis of different phytoconstituents. Among the eight different extracts, seven compounds were detected in ethanol extract,

methanol (six), acetone (six), aqueous (three), ethyl acetate (two), chloroform (two) and no compound was detected in petroleum ether and n-hexane as given in Table 2. The presence or absence of secondary metabolites depends on the type of solvent used for extraction. A similar type of work had been carried out on *Chlorella vulgaris* [21], *Ulva lacuta, Caulerpa racemosa, Sargassum wightii, Padina tetrastomatica, Gracilaria corticata, Acanthophora spicifera* [22], *Chlorococcum humicola* [23].

3.3. Quantitative analysis

The quantitative estimation is carried out to quantify the amount of secondary metabolites present in the crude seaweed powder. In the present research flavonoids (22.94±0.001 mg/g) are present in higher concentration, as compared to tannins (10.93±0.04 mg/g), and phenols (5.93±0.04 mg/g) as shown in Table 3. A similar type of result had been reported on *Acanthophora spicifera* [24] and a similar type of work had been carried out on *Dictyota dichotoma* [25].

Table 3: Quantitative analysis of secondary metabolites

Secondary metabolites	mg/g±SD
Flavonoids	22.94 ± 0.001
Tannins	10.93 ± 0.04
Phenols	5.93 ± 0.04

3.4. Biochemical Analysis

Red seaweed contains high carbohydrate content as compared to brown and green seaweed [26]. In the present biochemical analysis, carbohydrates are present in higher concentration (433.66±2.17 mg/g), as compared to proteins (19.627±0.33 mg/g), and lipids (50.33±1.52 mg/g) as shown in Table 4. The high carbohydrate content in red seaweed is due to high phycocolloid content present in their cell walls [27].

Table 4: Biochemical composition present in *Ahnfeltia plicata* extract

Secondary metabolites	mg/g±SD
Carbohydrates	433.66±2.17
Protein	19.627±0.33
Lipid	50.33±1.52

3.5. Estimation of Photosynthetic Pigments

The photosynthetic pigments analysis revealed the presence of chlorophyll a (0.8174±0.01ug/ml), chlorophyll b (0.4368±0.012ug/ml), total chlorophyll (1.2540±0.05ug/ml), Carotenoids (0.033±0.002 µg/g) and fucoxanthin (0.6±0.03µg/g) as shown in Table 5. A similar type of findings has been reported on *Codium adhaerens, Sargassum wightii* and *Acanthophora spicifera* [24], Chlorophyta, Phaeophyta and Rhodophyta species [28].

Table 5: Chlorophyll a, Chlorophyll b, Total Chlorophyll, Carotenoids, Fucoxanthin content present in *Ahnfeltia plicata* extract

Pigments	Content±SD
Chlorophyll a (ug/ml)	0.8174±0.01
Chlorophyll b (ug/ml)	0.4368±0.012
Total Chlorophyll (ug/ml)	1.2540±0.05
Carotenoids (µg/g)	0.033±0.002
Fucoxanthin(µg/g)	0.6±0.03

3.6. Phycobillins Estimation

The current Phycobilins estimation showed the presence of Allophycocyanin (10±0.25µgml^{-1}) which is maximum among C-phycocyanin (7±0.15µgml^{-1}) and C-phycoerythrin (4±0.2µgml^{-1}) as shown in Table 6**.** A similar type of work had been carried on cyanobacteria [29] and red seaweeds [27].

Table 6: Phycobillins Composition present in *Ahnfeltia plicata*

Phycobillins	(µg ml^{-1})±SD
C-phycocyanin (µg ml^{-1})	7± 0.15
AlloPhycocyanin (µg ml^{-1})	10±0.25
C-phycoerythrin (µg ml^{-1})	4 0.2

3.7. Antioxidant Activity

Table 7 reveals the maximum antioxidant activity of ethanolic extract of seaweed as compared to methanolic extract. Antioxidant activity is due to the presence of flavonoids, tannins and phenols [30-32]. The ethanolic extract shows free radical scavenging activity IC_{50} value at (2 mg/ml) whereas methanolic extract shows IC_{50} value at (15.625 mg/ml).

Table 7: Shows the Free Radical scavenging activity of *Ahnfeltia plicata* extract

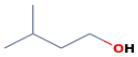
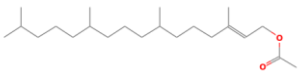
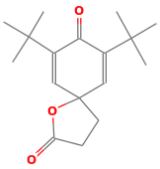
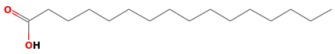
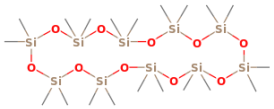
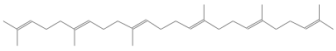
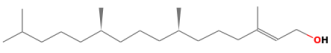
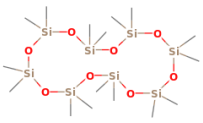
Extract	IC_{50} (mg/ml)
Methanol	15.625
Ethanol	2.0

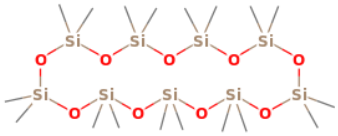
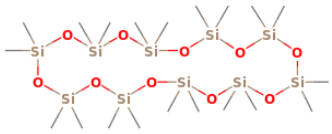
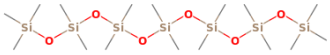
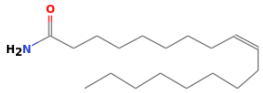
3.8. GC-MS ANALYSIS

The GC-MS analysis of *Ahnfeltia plicata* ethanolic extract shows 20 peaks indicating the presence of 20 different bioactive components. The NIST library was used to

identify and characterized the phytocompounds present in it. Out of which the maximum percent area was recorded in Ethane, 1, 1- diethoxy (31.03%), Naphthalene (8.21%), 1-Butanol, 3-methyl (7.99%), Tetradecane (6.6%), Squalene (6.05%) as shown in Table 8. 1, 1-diethoxy ethane is used as a flavoring agent in distilled beverages [33]. 3-methyl, 1-Butanol had an application in the purification of Nucleic acid [34]. Squalene is widely used in cosmetical formulations like moisturizing creams, makeup, and hair products [35]. Squalene is used for stimulating the immune response to increase the patient response to the vaccine [36].

Table 8: Retention time, Area %, Name and Structure of compound present in *Ahnfeltia plicata* extract detected by GC-MS Analysis

Peak #	Retention Time	Area %	Name	Structure
1	2.334	31.03	Ethane, 1,1-diethoxy-	
2	2.469	7.99	1-Butanol, 3-methyl-	
3	15.903	8.21	Naphthalene	
4	16.696	3.47	Dodecane	
5	23.656	6.6	Tetradecane	
6	29.92	4.98	Hexadecane	
7	33.216	3.04	Heptadecane	
8	35.595	2.66	Octadecane	
9	36.37	1	Phytol, acetate	
10	37.849	2.35	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	
11	38.558	3.3	n-Hexadecanoic acid	
12	38.789	2.46	Cyclodecasiloxane, eicosamethyl-	
13	39.034	4.6	Hexacosane	
14	39.239	6.05	Squalene	
15	40.743	2.43	Phytol	
16	40.857	2.46	Cyclooctasiloxane, hexadecamethyl-	

17	41.865	0.64	Cyclononasiloxane, octadecamethyl-	
18	42.119	2.93	Cyclodecasiloxane, eicosamethyl-	
19	42.628	1.71	Heptasiloxane, hexadecamethyl-	
20	43.347	2.09	9-Octadecenamide, (Z)-	

4. CONCLUSION

From the present research, it can be concluded that *Ahnfeltia plicata* is a rich source of bioactive compounds identified through preliminary phytochemicals analysis qualitative, quantitative and biochemical which were further confirmed by GC-MS analysis. They are abundant sources of carbohydrates, protein, flavonoids, Phycobiliprotein and also show higher antioxidant activity. However, the identification and isolation of major components of seaweed will be beneficial to manufacturers for the identification and selection of raw material for the development of a novel product in the field of pharmaceuticals, Nutraceuticals and cosmeceuticals industry.

5. Acknowledgement

The author is grateful tothe Principal and Botany Department of Ramnarain Ruia Autonomous College, Matunga (E), Mumbai, Maharashtra for providing the lab facility to carry out the research work and Botanical Survey of India, Coimbatore- 641003, Tamil Nadu for seaweeds authentication.

6. REFERENCES

1. Ito K, Hori K. *Food Rev. Int,* 1980; **5:**101-144.
2. Hosokawa M, Okada T, Mikami N, Konishi I, Miyashita K. *Food Sci. Biotechnol,* 2009; **18(1):**1-11.
3. Gonzalez R, Rodriguez S, Romay C, Ancheta O, et al. *Pharmacol Res,* 1999; **39:**55-59.
4. Zeliha D, Ferda F, Yilmaz K, Ulku N, et al. *J. Serb. Chem. Soc*, 2009; **74(6):**619-628.
5. Padula M, Bioteux S. *Braz. J.Med. Biol Res,* 1999; **32:**1063-1071.
6. Kumari P, Kumar M, Gupta V, Reddy CRK, et al. *Food Chemistry,* 2010; **120:**749-757.
7. Maggs CA and Pueschel CM. *J Phycol,* 1989; **25:**333-351.
8. Chapman VJ, Chapman DJ. Seaweeds and their uses. 3rd edn. London: 1980. p. 334.
9. Sukhoverkhov S V, Kadnikova I A, Podkorytova AV. *Appl Biochem Micro*, 2000**; 36:**201-203.
10. Kokate C K. A text book of Practical pharmacognosy. 5th ed. New Delhi: Vallabh Prakashan; 2005.P. 107- 111.
11. Harborne J B. Phytochemical method: A Guide to Modern technique of Plant Analysis. 3rd ed. New York: Chapman and Hall; 1998.
12. Sadasivam S, Manickam A. *In Biochemical methods for Agricultural sciences*. Wiley Eastern Limited, New Delhi. 1992; 6-7, 11-12.
13. Boham BA, Kocipai-Abyazan R. *Pacific Science,* 1974; **48:**458-463.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. *J.Biol. Chem,*1951; **193:**265.
15. Folch J, Lees M, Stanley GHS. *J. Biol. Chem,* 1957; **226:**497-509.
16. Arnon DI. *Plant Physiol,* 1949; **2:**1-15.
17. Kirk JTO, Allen RL. *Biochem. Biophysics Res. J,* 1965; **27:**523-530.
18. Seely GR, Duncan MJ,Vidaver WE. *Marine Biology*, 1972; **12:**184-188.
19. Pande J, Dhanki A, Padalia H, Chanda S. *The Pharma Innovation Journal,* 2018; **7(6):**78-86.
20. Mandlik R, Naik S, Tatiya A. *Int. J. Res Ayurved Pharm,*2014; **5(4)**:540-546.
21. Bhuvana P, Anuradha V, Syed M, Suganya V et al. *Bioscience Discovery,* 2018; **9(2):**244-250.

22. Thillaikkannu T, Mohan B, Kathiresan S. *International Research Journal of Pharmacy,* 2012; **3(7):**261-265.
23. Kavitha J, Palani S. *World Journal of Pharmacy and Pharmaceutical Sciences,* 2016; **5(6):**1154-1167.
24. Seenivasan R, Rekha M, Indu H, Geetha S. *India. Journal of Applied Pharmaceutical Science*, 2012; **2(10):**159-169.
25. Mohammed D, Taha E, Fatma W. *International Journal of Engineering Development and Research,* 2016; **4(2):**674-678.
26. Dhargalkar VK, Jagtap TG, Untawale. *Indian J. Mar. Sci,*1980; **9(4):**297-299.
27. Suresh KG, Marisamy K, Senthilkumar N. *International Journal of Recent Scientific Research,* 2017; **8(12):**22399-22406.
28. Vimala T, Poonghuzhali TV. *International Journal of Science and Research,* 2013; **4(10):**1850-1854.
29. Mathivannan S, Ekumalai S, Rajest K. *Agrt.Isrj.Org,* 2015; **4(8):**1-4.
30. Cody V, Middleton E, Harborne JB, Baretze A. Plant flavonoids. In: *Biology and medicine II: Biochemical, cellular and medicinal properties*. Alan R. LissInc, New York: 1988. P. 330.
31. Koleckar V, Kubikova K, Rehakova Z, Kuca K, et al. *Mini Rev. Med. Chem,* 2008; **8:**436-447.
32. Chung KT, Wong TY, Wei CI, Huang YW, et al. *Crit. Rev. Food Sci. Nutr,*1998; **38:**421-464.
33. https://pubchem.ncbi.nlm.nih.gov/compound/Acetal#section=Human-Metabolite Information.
34. Green M, Sambrook J. Molecular Cloning: A Laboratory Manual.Fourth Edition 2012.
35. Huang Z R, Lin YK, Fang JY. *Molecules,* 2009; **14:**540-554.
36. Fox CB. *Molecules,*2009; **14:**3286-3312.