iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 13 **Issue:** III **Month of publication:** March 2025

DOI: https://doi.org/10.22214/ijraset.2025.67287

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 13 Issue III Mar 2025- Available at www.ijraset.com

Phytochemical screening, Antioxidant Activity and GC-MS analysis of marine alga *Spatoglossum asperum*(j.agardh)-Dictyotaceae from Kunkeshwar coast of Devgad district

Nilesh Kadam[1], Sunil Shankhadarwar[2]
[1, 2]*Department of Botany, Ramnarain Ruia Autonomous College, Matunga, Mumbai – 400 019, Maharashtra, India.*

Abstract: ***The present investigation aimed to assess and evaluate secondary metabolites and antioxidant activity of marine alga Spatoglossum asperum. The brown alga was collected from Kunkeshwar coast of Devgad district, Maharashtra, authenticated, shade dried and grinded to powder. The primary phytochemical screening was carried out by extracting the alga with different polar and non polar solvents namely ethanol, methanol, ethyl acetate, petroleum ether, chloroform, n- Hexane, diethyl ether. The significant free radical scavenging activity against DPPH assay was examined for antioxidant property. Ethanolic extract obtained by Soxhlet assembly was subjected to GC-MS analysis to reveal the richness of its bioactive compounds. From the study, it can be concluded that Spatoglossum asperum serve as a potent and promising source for natural antioxidant lead molecules in cosmeceutical and pharmaceutical industry.***

Keywords: ***Secondary metabolites, Spatoglossum asperum, Phytochemicals, DPPH, GC-MS.***

I. INTRODUCTION

Algae are widespread almost everywhere on our planet and are economically, ecologically and biologically significant component comprised of diversified group of plants. Marine algae have developed defense strategies in order to survive and compete with rest flora under seawater which has further resulted in enormous diversity of compounds from different metabolic pathways.The nutritional value of algae and their potential use as functional ingredients in food can be assessed which would be a new opportunity to introduce seaweeds indirectly into human food chain as nutritional supplements [9]. Seaweeds valued as medicinal and rich in secondary metabolites are of immense medicinal value and have been enormously used in drug and pharmaceutical industry [1], [16].

These bioactive substances can showcase several nutraceutical and pharmaceutical behavior to defend from various diseases.

While algae species have already been used in cosmetic formulations, such as moisturizing and thickening agents, marine algae have not been explored as an asset in cosmeceuticals due to lack of utility as a primary active ingredient [2]. This study can be integrated with the purpose of identifying serviceable algae functions in practical cosmetic uses.

Antioxidant activity study can be utilized for the development of natural antioxidants as these compounds serve both as preservatives and protectors against oxidation of foods and cosmetics. The findings of current study might aim for the use of flavonoid and phenol rich compounds as natural antioxidants in different food and pharmaceutical products [24].

In this background, the present study aimed to assess and evaluate potentially bioactive secondary metabolites by phytochemical screening, GC MS analysis and antioxidant activity of marine alga *Spatoglossum asperum* J. Agardh.

II. MATERIALS AND METHODS

A. *Collection and Authentication of algal material*

The marine brown alga *Spatoglossum asperum* was collected from the Kunkeshwar coast of Devgad district, Maharashtra, India. The sample was authenticated from Botanical Survey of India, Coimbatore. The freshly collected seaweed was carefully rinsed with fresh water to remove epiphytes, sand and debris adhered. Later it was shade dried for 6-7 days till complete dryness, weighed, milled into a fine powder and stored in airtight containers for further studies.

B. Algal extract preparation

Ten gm of dried and powdered seaweed was successively extracted with 100ml of various solvents of increasing polarity such as petroleum ether, chloroform, n-hexane, diethyl ether, ethyl acetate, ethanol, methanol and water by cold maceration for 24 hrs (6 hrs on a rotary shaker at 100 rpm and 18 hours standing). The extracts were filtered using Whatman's filter No. 1, evaporated on a water bath, reconstituted by using suitable solvent to make up the final volume to 100 ml. The percentage of extractable value was calculated with reference to sample amount taken earlier as reported by Rakholiya K *et al*., 2011[18]. The obtained extracts were stored in a refrigerator for further phytochemical screening.

Determination of methanol soluble extractive value Five grams of dried powder was taken in 100 ml of methanol in a conical ask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. □erea□er, it was □ltered and the □ltrate was evaporated to dryness at 105°C till constant weight was obtained. □e percentage of extractable matter was calculated with reference to the sample taken

III. QUALITATIVE PHYTOCHEMICAL SCREENING

The phytochemical screening of different algal extracts was examined by a standard protocol as described by Kokate *et al.,* (2005) and Harborne (1998) [10], [12]. Phytochemical screening was carried out to identify the phytoconstituents such as alkaloids, phenols, terpenoids, steroids, tannins, saponins, flavonoids, coumarins, quinones and glycosides. General chemical reactions in these analyses determined the presence or absence of these compounds in the algal extracts tested which added to its potentiality as a bioactive principle. Similar phytochemical studies were reported in marine brown alga *Turbinaria ornate* by Gopalan Rajkumar and Periyakali Saravana Bhavan (2017) [8].

IV. QUANTITATIVE PHYTOCHEMICAL SCREENING

From the preliminary qualitative analysis, ethanol extract of *Spatoglossum asperum* revealed a maximum number of secondary metabolites. Hence, the ethanolic extract was used to pursue further quantitative study. The standard quantitative method of screening phenol, flavonoids and tannins, reveals the amount of phytochemical compounds present in the sample as reported by M. S. Leelavathi and Prasad M. P (2015) [13]. The standard protocol was used for estimation of phenol and tannins using the Folin - Ciocalteau method by Sadasivam and Manickam (1992) [19] and for flavonoids by Bohm and Kocipai-Abyazan (1994) [4].

V. DETERMINATION OF ANTIOXIDANT PROPERTY BY DPPH RADICAL SCAVENGING ACTIVITY

Free radical scavenging activity was carried out by the DPPH method as described by Sreejayan and Rao (1996) [21]. In this method ethanolic extract of brown alga *Spatoglossum asperum* was prepared in various concentrations from 100 to 900µg/ml, each one of the above concentrations was added to 1 ml of DPPH (0.004%). An equal amount of ethanol was added to the control. After 30 mins of incubation at 37˚C in darkness, the absorbance was read at 517 nm by using a spectrophotometer. The antioxidant activity was expressed in IC_{50} (mg/ml) and the percentage of inhibition was calculated by using the formula as mentioned by Prasanth *et al.,* (2000) [17]. The experiment was performed in triplicates.

$$\% \text{ of Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test Sample})}{\text{Absorbance of Control}} * 100\text{mg/ml}$$

VI. GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC- MS) ANALYSIS

The 1µl of ethanolic extract of *Spatoglossum asperum* was injected by automated syringe in the split mode and analyzed by Shimadzu GCMS-QP2010 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 a carrier gas. The ionization energy of 70 eV was used for the electron ionization system. The initial oven temperature was programmed at 40º C for 3mins and later was raised to 150º C at the rate of 7º C/mins. The ion source temperature and injection temperature was 200 º C and the interface temperature was set at 250º C. Identification of various components present in the extract was done by comparison of retention indices and mass fragmentation patterns with those stored on the computer library along with published literature. NIST and Willey, Vandendool H. and Kratz P.D (1963) [23] online library sources were used for matching the identified compounds.

VII. RESULTS AND DISCUSSION

A. *Soluble Extractive Value*

The extractive values are significant to evaluate the secondary metabolites present in the crude drug and also aid in the estimation of specific constituents soluble in a particular solvent [18]. The higher yield was depending upon the solvent type selected which dissolves more of a particular compound. The extractive value of *Spatoglossum asperum* in different polar and non-polar solvents is shown in Table 1. The maximum extractive value was 1.242 % found in the aqueous water solvent while the minimum 0.696 % was in n-Hexane extract. The ethanolic extractive value was found to be 1.104%.

Table 1. Extractive values of *Spatoglossum asperum* in different solvents.

Sr.No	Solvent	Extractive value (% Yield)
1	Ethanol	1.104 %
2	Methanol	0.896 %
3	Ethyl acetate	0.788 %
4	Petroleum ether	0.746 %
5	Chloroform	0.762 %
6	n-Hexane	0.696 %
7	Diethyl ether	0.862 %
8	Aqueous extract	1.242 %

B. *Qualitative Phytochemical Analysis*

Preliminary phytochemical screening of *Spatoglossum asperum* for the presence of ten different phytochemicals was tested in eight different solvent extracts. Among the ten different extracts, seven compounds were detected in ethanol extract, methanol (six), aqueous (five), diethyl ether (four), ethyl acetate (three), petroleum ether (two), n Hexane (one) as given in Table 2. The results showed that quinones and glycosides did not show their positive presence in any of the extracts tested. The presence or absence of secondary metabolites depends on the type of solvent used for extraction. A similar type of work has been reported by Kavitha and Palani 2016, Thillaikkannu *et al.,* 2012 [11], [22].

Table 2. Qualitative phytochemical screening of *Spatoglossum asperum* in different solvent extracts.

Sr.No	Phytochemicals	Ethanol	Methanol	Ethyl acetate	Petroleum ether	Chloroform	n-hexane	Diethyl ether	Aqueous
1	Alkaloids	++	++	++	+	-	-	+	+
2	Terpenoids	+	+	-	-	-	-	-	-
3	Steroids	-	-	-	-	-	-	-	-
4	Tannins	++	+	-	-	-	-	+	+
5	Saponins	+	-	-	-	-	-	-	+
6	Flavonoids	+++	++	++	+	+	+	++	+
7	Phenols	+	+	+	-	-	-	-	-
8	Coumarins	+	+	-	-	+	-	+	+
9	Quinones	-	-	-	-	-	-	-	-
10	Glycosides	-	-	-	-	-	-	-	-
(+++ more amount); (++ moderate amount); (+ present); (- negative)									

C. *Quantitative phytochemical screening*

The ethanolic extract of *Spatoglossum asperum* was used for quantitative estimation of secondary metabolites. Flavonoids, was found to be in higher concentration (33.4 ± 0.2 % mg/g) as compared to phenols (8.92 ± 0.3 % mg/g) and tannins (6.96 ± 0.1 % mg/g) as shown in Table 3. A similar type of result has been reported by Seenivasan *et al* 2012 [20] on *Acanthophora spicifera*.

Table 3. Quantitative phytochemical screening of Spatoglossum *asperum.*

Sr.No	Secondary metabolites	mg/g ± SD
1	Flavonoids	33.4 ± 0.2
2	Phenols	8.92 ± 0.3
3	Tannins	6.96 ± 0.1

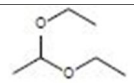
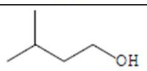
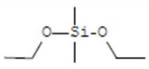
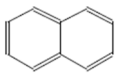
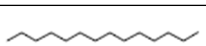
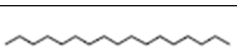
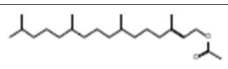
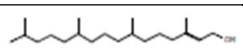
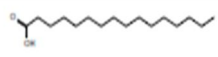
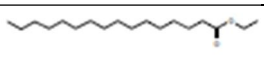
D. Antioxidant activity by DPPH Radical Scavenging Activity

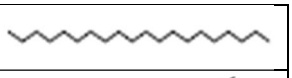
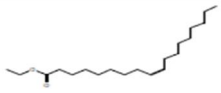
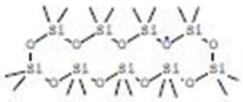
The antioxidant activity is due to the presence of Flavonoids, tannins and phenols present in extract as reported by Chung *et al.*, 1998; Cody *et al.,* 1988 [5-6]. The ethanolic extract showed free radical scavenging activity of IC_{50} value at 160 µg/ml concentration.

E. Gas Chromatography-Mass Spectrometry (GC- MS) Analysis

In this present study, the GC-MS analysis indicated the presence of bioactive components in the ethanolic extract of *Spatoglossum asperum*. 16 compounds were observed with retention time (RT), and peak area value (%) as presented in Table 4. The results revealed the presence of major phytocompounds like Ethane, 1,1-diethoxy (20.82%, R.T: 2.337), n-Hexadecanoic acid (11.93%, R.T: 38.558), Naphthalene (10.96%, R.T: 15.899), 1,4-Eicosadiene (7.46%, R.T: 37.152), Hexadecanoic acid, ethyl ester (7.03%, R.T: 38.984). Ethane, 1,1-diethoxy is used as flavoring agent in distilled beverages as reported by PubChem. n-Hexadecanoic acid finds use as antibacterial & antifungal agent as reported by Chandrasekaran M *et al.,* (2011) [5], Hexadecanoic acid, ethyl ester has antibacterial & antifungal property as mentioned by Babu *et al.,* (2014) [3] in biological activity of *Ulva lactuca.*

Table 4: GC-MS analysis of phytochemicals identified from the ethanolic extract of *Spatoglossum asperum.*

Sr.No	R.T	Name of the Compound	Area%	Chemical Structure
1	2.337	Ethane, 1,1-diethoxy-	20.82	
2	2.479	1-Butanol, 3-methyl-	5.43	
3	2.864	Silane, diethoxydimethyl-	2.72	
4	15.899	Naphthalene	10.96	
5	16.693	Dodecane	3.8	
6	23.66	Tetradecane	6.03	
7	29.929	Hexadecane	6.34	
8	35.599	Heptadecane	3.63	
9	36.37	Phytol, acetate	4.81	
10	36.835	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	4.13	
11	37.152	1,4-Eicosadiene	7.46	
12	38.558	n-Hexadecanoic acid	11.93	
13	38.984	Hexadecanoic acid, ethyl ester	7.03	

14	39.04	Eicosane	1.58	
15	41.341	Ethyl Oleate	2.31	
16	42.071	Cyclononasiloxane, octadecamethyl-	1.02	

Similar type of work on *Spatoglossum asperum* reported by Movahhedin *et al.,* 2018, focused on the extract isolated by petroleum ether, dichloromethane and methanol. In terms of GC identified chemical components, our results varied with the previous studies of *Spatoglossum asperum* on extraction methods by different solvents. The activity of marine alga may vary according to the type of extraction methods, solvents used, species, geographical location, seasonality, collection procedures (Manilal, *et al.,* 2009) [14].
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edema, Anemia, Respiratory failure, Drowsiness, Diarr

VIII. CONCLUSION

The present study of *Spatoglossum asperum* on phytochemical screening, antioxidant activity and GC-MS analysis provides valuable information about richness of the marine brown alga concerning its diverse phytochemicals like flavonoids, phenols, tannins, alkaloids, coumarins and its antioxidant property of endogenous defense mechanism as a protection against oxidative stress due to extreme level of environmental conditions. The investigation findings concluded that the stronger extraction capacity of ethanol could have enabled to produce several active secondary metabolites responsible for several biological activities and hence proved as potent seaweed for the development of pharmaceutical compounds. These secondary metabolites might be utilized in near future for the expansion of traditional medicines and further exploration needs to elute novel active compounds from the seaweed which may pave a new way to treat many fatal diseases that need to be conquered upon. Further, it is suggested for isolation, purification and characterization of individual bioactive compounds from *Spatoglossum asperum* to study their unique active principles.

ACKNOWLEDGMENT

The authors are grateful to the Principal, Ramanarain Ruia Autonomous College, Matunga (E), Mumbai, Maharashtra for providing basic research facilities. The authors also gratefully acknowledge Botanical Survey of India (BSI), Coimbatore, India, for authentication of the seaweed, *Spatoglossum asperum*.

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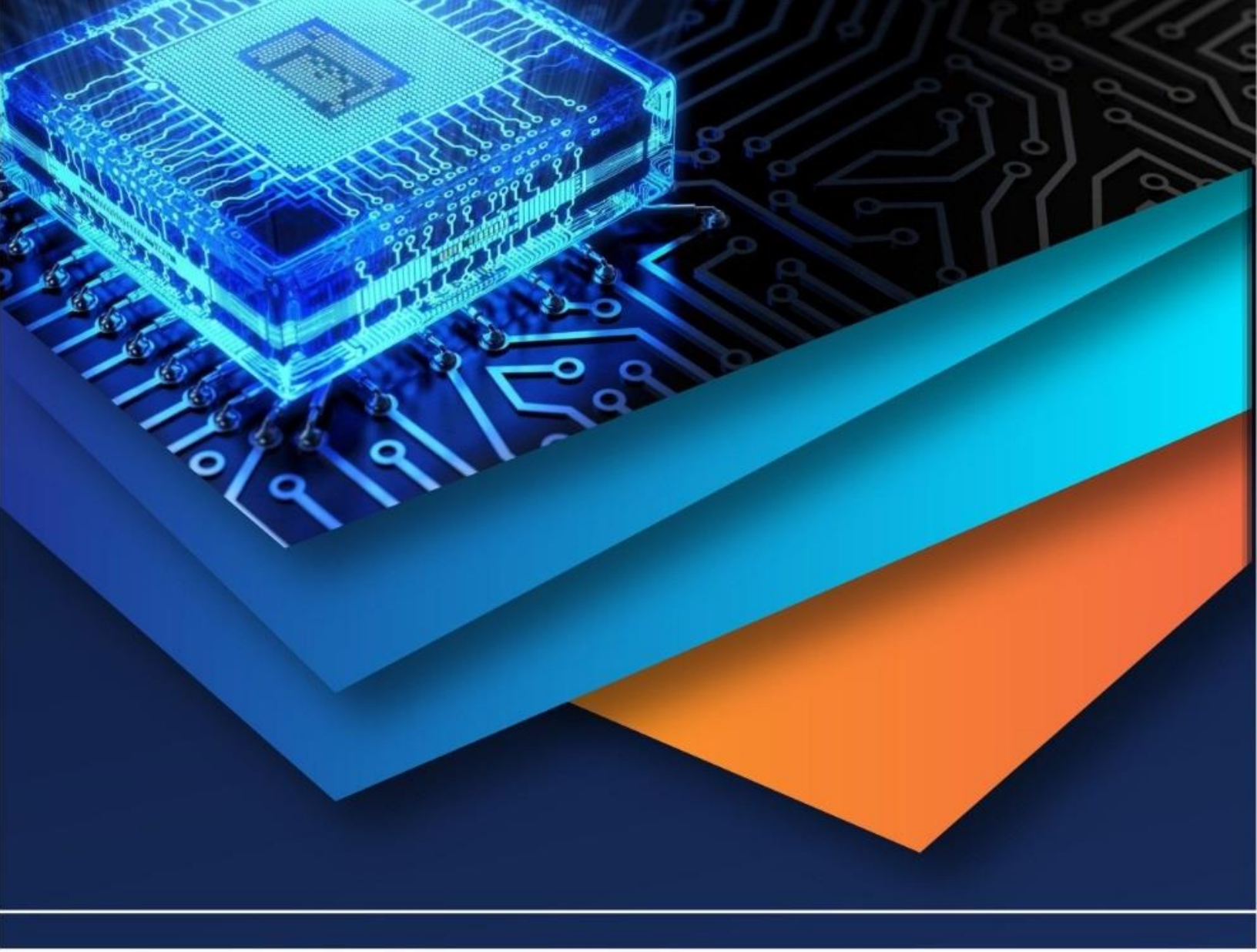

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