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IDENTIFICATION AND QUANTIFICATION OF POTENTIAL BIOACTIVE COMPOUNDS FROM SEAWEED OF *CHAMPIA COMPRESSA*

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Keywords:

Champia compressa, Phytochemical, Seaweeds, FT-IR, GC-MS

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ABSTRACT: The present investigation was carried out to analyze the bioactive metabolites of red seaweed *Champia compressa* collected from Mandapam, Ramanathapuram district, Tamil Nadu, India. The collected sample was evaluated for qualitative and quantitative phytochemical screening analysis using five different solvents methods. The preliminary qualitative analysis of *Champia compressa* solvent extracts revealed the presence of flavonoids, tannins, steroids, cardiac glycosides, terpenoids, coumarins, alkaloids, saponins, and phenols. Further quantitative phytochemical analysis was performed using standard procedures that revealed the presence of the highest phenol content (506.67 mg GAE/g) found in aqueous extract and the lowest in chloroform extract (8.33 mg GAE/g). Similarly, alkaloid content (34.09 mg AE/g) was highest in ethyl acetate extract and lowest (15 mg AE/g) in aqueous extract. The highest steroid content (85.88 mg BSE/g) was observed in hexane extract, whereas the lowest (3.69 mg BSE/g) in chloroform extract, however terpenoid (30.25 mg LE/g) was higher in methanolic extract, and the least (3.38 mg LE/g) found in aqueous extract respectively. The FTIR characterization represents the functional groups of fourteen major peaks and sixteen bioactive compounds observed from the GC-MS spectrum from the methanolic extract. Hence these results confirmed the presence of potentially bioactive compounds of *Champia compressa* possibly used to develop novel drugs for treating various diseases.

INTRODUCTION: Seaweeds are now understood to be a rich source of bioactive chemicals, which are credited with being rich in bioactive substances like polysaccharides, polyunsaturated fatty acids, pigments, polyphenols, minerals, and plant hormones that promote development.

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In addition, plant components such as flavonoids, alkaloids, lectins, laminarin, fucoidan, galactan, alginate, and protein are compounds made of chlorophylls, carotenoids, and substantial involvement in the global fight illnesses with a high mortality rate and pathogens [1, 2].

These distinctive phytochemicals have significant biological effects and a wide range of pharmacological potential, including antibacterial, anti-inflammatory, anti-arthritic and anti-proliferative actions. Seaweeds are also employed in the generation of novel pharmaceuticals [3, 4].

However these activities often originate from a range of secondary metabolites from macro algae-derived compounds such as alkaloids, flavonoids, steroids, glycosides, terpenes, tannins, fatty acids, and phenol compounds [5, 6, 7]. Among seaweeds, red seaweeds are harvested and consumed as raw or dried food used especially in soups and salads between different places. These red seaweeds are non-toxic and are beneficial to marine aquarium grown in a controlled manner. In the present study *Champia compressa* red seaweed belonged to the Champiaceae family was chosen because only petite data are available in the literature related to bioactive compounds. Further, his attempt was undertaken to examine the qualitative and quantitative phytochemical constituents of five different polar to non-polar solvent extracts of *Champia compressa* was analyzed by FT-IR and Gas Chromatography and Mass Spectrometry (GC-MS) to provide scientific information for generating new drug designs for future research.

MATERIALS AND METHODS:

Sample Preparation: *Champia compressa* was collected Rameshwaram coast, Ramanathapuram, Tamil Nadu, South India. The collected sample was authenticated by the Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India.

Processing of Collected Sample: The collected seaweeds were initially rinsed with seawater to eliminate dirt and debris along with epiphytes, sand particles, and shells. Later, they were washed with running tap water followed by distilled water and dried in dark conditions. The resulting dried material was coarsely powdered (passing through a 40-size sieve) and utilized for further pharmacognostic and phytochemical studies.

Preparation of Seaweed Extracts using the Different Solvent Method: The shade-dried *Champia compressa* were powdered using a milling machine and further subjected to Soxhlet extraction (Borosil, Mumbai, India) using five different solvents, namely Methanol, Ethylacetate, Aqueous, Chloroform and Hexane. The powdered samples were subjected to a 20gm thimble using handmade filter paper. Later, *Champia compressa*, the filled thimble was carefully placed within the extractor chamber and poured with five chosen dissimilar solvents as a 1:10 ratio. In a heating mantle, the reservoir round bottom flask was excited to 60°C. Each sample was run at least 15 refluxes to get good quality seaweed solvent extracts. The resultant solvent extracts were condensed using a rotary evaporator (Buchi, Bangalore, India) under a condensed temperature under vacuum conditions. Finally, the precipitant was collected in a glass container and stored under -20°C for further analysis.

Qualitative Analysis of Phytochemical Screening: Preliminary phytochemical analysis was carried out to identify secondary metabolites present in polar to non-polar solvent extracts of *Champia compressa* using a standard protocol (Pang *et al.* 2017; Muttakin and Zulfajri 2020) [8, 9].

Quantitative Phytochemical Screening of *Champia compressa*:

Determination of Total Phenol: According to the Folin-Ciocalteau colorimetric method (Singleton and Rossi, 1965) solvent extracts were used for the determination of the total phenolics spectro-photometrically. The obtained five different solvent extracts of *Champia compressa* (200 µL) were introduced into screw cap test tubes, and 1.0 mL of Folin-Ciocalteau reagent (1:1 with water) and 1.0 mL of sodium carbonate (7.5%) were added. The corresponding tubes were vortexed and incubated for 2 hours, and the absorbance was read at 726 nm using a spectrophotometer (Beckman, The USA). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of dry material [10].

Determination of Alkaloids: The alkaloids were determined using *Champia compressa* seaweed, in which 5 gm was taken and placed in a 250ml beaker and 200ml of 10% acetic acid (CH3CO2H) in ethanol (C2H5OH) was added. The concoction was enclosed and allowed to stand for 4 hours at 25°C at room temperature. The tested sample was filtered using filter paper No. 42, and then filtrate was concentrated in a water bath until it reached the original quantity. Following that, concentrated NH_4OH was added drop-wise until precipitation was complete. The mixture was permitted to resolve, and then collected precipitate was weighed on filter paper and washed with dilute

NH_4OH. Finally the precipitate in addition to the alkaloid, was dried and weighed. The percentage of alkaloids was calculated by difference.

Determination of Terpenoids: Total terpenoids were determined using the method proposed by Ghorai *et al* (2012), Briefly, l ml of methanol was dissolved, a known amount of *Champia compressa* (20 mg) was added with 1.5 ml chloroform in every 2 ml micro-centrifuge tube. The sample mixture vortex thoroughly and allowed it to stand for 3 minutes. Subsequently, 100 µl of concentrated sulphuric acid (H_2SO_4) was added to every 2 ml micro-centrifuge tube. Then the assay tube was incubated at room temperature (~30°C) for 1.5-2 hours under dark conditions.

After incubation, reddish-brown precipitation will be produced in each analyzed micro-centrifuge tube. Then all supernatant of the reaction mixture liquid was decanted without disturbing the precipitation. Later, 1.5 ml of 95% (v/v) methanol was added and vortexed thoroughly until all the precipitation completely dissolved in methanol.

Then the samples were transferred from assay tube to colorimetric cuvette, and absorbance read at 538 nm spectrophotometrically against 95% (v/v) methanol was served as blank. The total terpenoids were calculated using a standard curve from the blank-corrected wavelength 538 nm of the Linalool standard [11, 12].

Determination of Steroids: The total steroids were determined using (Liebermann-Burchard Reaction) in which 200 mg of *Champia compressa* seaweed was dissolved in 10 ml of chloroform and filtered. After that 2 ml of filtrate was taken, and 2 ml of acetic anhydride along with H_2SO_4 was added. The presence of blue/green rings indicated the occurrence of steroids.

FT-IR Analysis of *Champia compressa*: FT-IR studies have been followed by Diem method in which dried powder of different solvent extracts of *Champia compressa* was used for FTIR analysis. The dried solvent extract powder 10 mg was encapsulated in 100 mg KBr pellet. The IR-spectra region between 4000-500 cm-1 was recorded at room temperature on a Bruker ATR series FTIR spectrometer. The sharp band frequencies were accurate to 0.01 cm^{-1}.

GC-MS Analysis of *Champia compressa*: The Gas Chromatogram Shimadzu GC-2010 Plus was equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC-5 column (250µ I.D., 0.25µ film thickness). A split injection was used for sample introduction , and the split ratio was set to 10:1. The oven temperature program was programmed to start at 35°C and seize for 2 minutes, then ramp at 20°C per minute to 450°C and hold for 5 minutes. The helium carrier gas was set to a 2 ml/minute flow rate (constant flow mode). The mass spectrum was the connection with a capillary column metal quadrupole mass filter pre-rod mass spectrometer operating in electron ionization (EI) mode with software GCMS solution ver. 2.6 were used for all analyses.

Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanned from m/z 25 to m/z 1000 at 0.3 seconds per scan with a 0.2-second inter-scan delay. High-resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 1000 at 1 second per scan. The identified compounds matched their recorded spectra with the data bank mass spectra of the NIST library V11 provided by the instrument's software. GC / MS Metabolomics. The database was used for the similarity search with the retention index.

RESULT AND DISCUSSION:

Qualitative Analysis of Phytochemicals in Different Solvent Extracts from *Champia compressa*: The phytochemical screening from the chloroform extract of *Champia compressa* showed the presence of coumarins, terpenoids, steroids, alkaloids, and phenols. However, the hexane extract of *Champia compressa* revealed the presence of phytochemical constituents like steroids, alkaloids, and phenols. In the case of methanolic and aqueous extract of seaweed flavonoids, tannins and terpenoids are also present with the same compound obtained in hexane extract. Still, the absence of terpenoids is noted, especially in aqueous extract. In contrast, ethyl acetate seaweed extract of *Champia compressa* showed the presence of nine compounds as compared to other solvents methods. The presence of these bioactive compounds in different seaweed

extracts may be the source of various biological activities (antiviral, anti-inflammatory, and anticoagulant) [13]. The qualitative phytochemical screening analysis depicted the presence of phenol, terpenoids, saponins, alkaloids, and steroids as commonly present in chloroform, hexane, aqueous, methanol, and ethyl acetate extracts of *Champia compressa* . In contrast, anthraquinone was absent in all the extracts tested, as shown in **Table 1.**

TABLE 1: QUALITATIVE ANALYSIS OF PHYTOCHEMICALS IN DIFFERENT SOLVENT EXTRACTS FROM *CHAMPIA COMPRESSA*

Constituents	Chloroform	Hexane	Ethyl Acetate	Methanol	Aqueous
Coumarins	+	-	+	-	-
Flavonoids	-	-	+	+	+
Tannins	-	-	+	+	+
Anthraquinones	-	-	-	-	-
Steroids	+	+	+	+	+
Cardiac Glycosides	-	-	+	-	-
Terpenoids	+	-	+	+	-
Alkaloids	+	+	+	+	+
Saponins	-	-	+	+	-
Phenols	+	+	+	+	+

Quantitative Analysis of *Champia compressa*: The quantitative phytochemical analysis of *Champia compressa* revealed the highest phenol content in aqueous extract and lowest in chloroform extract was observed. The alkaloid content was found to be the highest in ethyl acetate extract and lowest in aqueous extract. However, the highest steroid content was observed in hexane extract and the least in chloroform extract. In contrast, terpenoid content was observed to be higher in methanolic extract and lowest in aqueous extract respectively **Table 2.**

TABLE 2: QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS IN DIFFERENT SOLVENT EXTRACTS FROM *CHAMPIA COMPRESSA*

Constituents / Extracts	Phenol (Mg GAE/g)	Alkaloid (Mg AE/g)	Steroids (Mg BSE/g)	Terpenoids (Mg LE/g)
Chloroform	8.33 ±0.04	31.36 ±0.03	3.69 ±0.01	5.88 ±0.09
Hexane	60.00±0.02	33.64 ±0.03	85.88±0.05	12.38±0.10
Ethyl Acetate	28.00 ±0.06	34.09 ±0.03	60.31 ±0.03	9.92±0.03
Methanol	457.33±0.04	25.91 ±0.04	11.00 ±0.03	30.25±0.02
Aqueous	506.67±0.12	15.00 ±0.06	12.25 ±0.03	3.38 ±0.05

These bioactive compounds like alkaloids, phenols, and terpenoids have various health-promoting effects with medicinal value. Numerous phytochemical studies have been reported in the coastal area of marine red algae. Alkaloids are found to have cytotoxic, antimicrobial and antispasmodic properties and steroids from marine algae have therapeutic values Phenols compounds were reported in *Dictyota dichotoma* brown algae which is responsible for antioxidant activity [14]. Therefore phytochemical screening of *Champia compressa* is essential for identifying novel sources of bioactive compounds in medicinal and industrial aspects. Hence, it is very important to initiate red algae to screen secondary metabolites.

GC-MS Analysis: Through GC-MS analysis, methanolic extract of *Champia compressa* was identified initially and nearly sixteen chromatogram peaks were obtained, namely piperazine, delta-selinene, caryophyllene, 3-heptadecene(z), tetradecanoic acid, phytol, acetate, oxirane, hexadecanal, dotriacontane, tetracontane, hexatriacontane, tetratriacontane, pentatriacontane, squalene, pentacosane, and fucosterol by comparing with mass spectra of 135 constituents with NIST library. The retention time, area percentage, compound name, molecular formula, and molecular weight are depicted in **Table 3,** and the chromatogram report was observed in **Fig. 1.** However, these compounds exhibited a wide range of pharmacological activities, wherein the largest peak area was observed for compound 1-Fucosterol and the highest molecular weight was tetracontane, respectively.

The results of present investigations concur with previous studies by Raubbin R. S *et al.,* which reported tetracontane and tetradecanoic acid, identified from Hypnea Flagelliformis [15]. Tetracontane and 1- Fucosterol compounds exhibit a wide range of antimicrobial properties [16, 17, 18]. In the same study, squalene was shown to possess better antimicrobial and antioxidant properties [19].

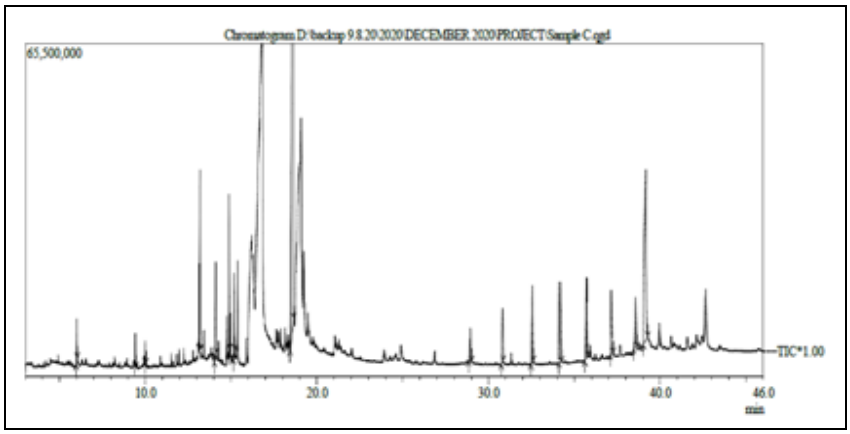
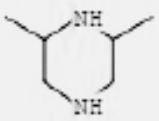
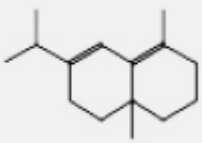
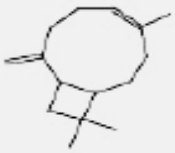
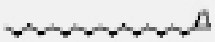
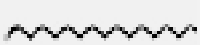
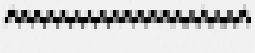
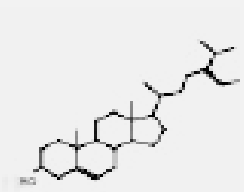


FIG. 1: PEAK AREA PERCENTAGE OF GC-MS IN METHANOLIC EXTRACT OF *CHAMPIA COMPRESSA*

TABLE 3: PHYTOCOMPONENTS IDENTIFIED BY GC-MS ANALYSIS OF METHANOLIC EXTRACT OF *CHAMPIA COMPRESSA*

S. no.	RT	Name of the Compound	Molecular Formula	Molecular Weight (g/mol)	Peak (Area %)	Molecular Structure	Reported Bioactivity
1	5.999	Piperazine	$C_6H_{14}N_2$	114	1.02		Anti microbial activity
2	9.415	delta.-Selinene	$C_{15}H_{24}$	208	0.81		Anti microbial activity
3	9.997	Caryophyllene	$C_{15}H_{24}$	204	0.53		Anti cancer, antioxidant, anti microbial, anti inflammatory activities
4	13.186	3-Heptadecene, (Z)-	$C_{17}H_{34}$	238	4.68		Anti microbial and anti tumour activity
5	14.111	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	3.31		Antioxidant, anti microbial activity
6	14.893	Phytol, acetate	$C_{22}H_{42}O_2$	338	3.97		antimicrobial, antioxidant, antinociceptive, antidiabetic activity
7	15.170	Oxirane	$C_{18}H_{36}O$	268	2.40		Anti microbial and anti oxidant activity
8	18.598	Hexadecanal	$C_{16}H_{32}O$	240	44.75		Anti bacterial activity
9	28.936	Dotriacontane	$C_{32}H_{66}$	450	1.78		Anti inflammatory activity

10	30.815	Tetracontane	$C_{40}H_{82}$	562	2.61		Anti oxidant and anti microbial activity
11	32.550	Hexatriacontane	$C_{36}H_{74}$	506	3.86		Anti microbial and anti cancer activity
12	34.170	Tetratriacontane	$C_{34}H_{70}$	478	4.24		Anti inflammatory activity
13	35.699	Pentatriacontane	$C_{35}H_{72}$	492	4.22		Anti microbial activity
14	37.160	Squalane	$C_{30}H_{62}$	422	3.24		Anti oxidant activity
15	38.563	Pentacosane	$C_{25}H_{52}$	352	3.06		Anti oxidant and anti microbial activity
16	39.165	Fucosterol	$C_{29}H_{48}O$	412	15.49		Anti inflammatory, Anti adipogenic activity

FTIR Analysis: The FTIR studies on methanolic extract of *Champia compressa* confirmed the presence of major peaks denoting phenol, alkane, alkene, carboxylic acid, nitro compound, isothiocyanate, and aliphatic ether compounds, and corresponding functional groups are depicted in **Table 4,** and FTIR spectral report was depicted in **Fig. 2.**

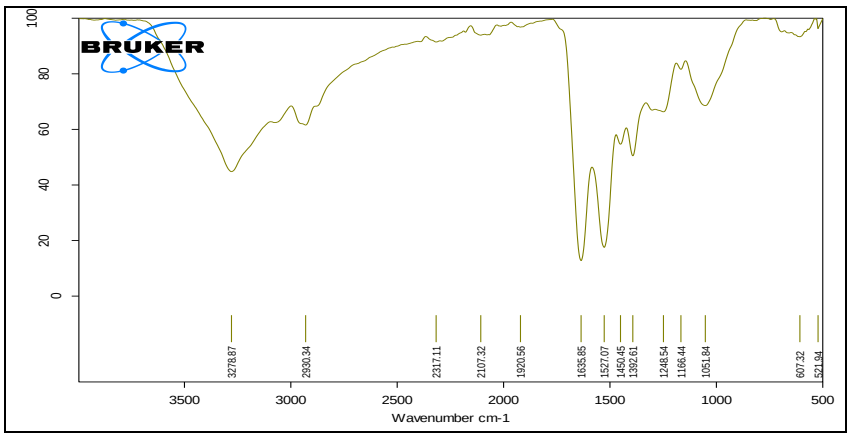


FIG. 2: FT-IR ANALYSIS OF METHANOLIC EXTRACT OF *CHAMPIA COMPRESSA*

TABLE 4: FT-IR SPECTRA ANALYSIS OF METHANOLIC EXTRACT OF *CHAMPIA COMPRESSA*

S. no.	Peak value	Corresponding Functional group
1	3278.87	O-H stretching, Carboxylic acid
2	2930	N-H stretching, Amine salt
3	2317.11	O=O=O stretching, Carbondi oxide
4	2107.32	N=C=S Stretching, Isothiocyanate
5	1920.56	C=C=C StretchingAllene
6	1635.85	C=N Stretching, Imine/oxime
7	1527. 07	N-O Stretching, Nitro compound
8	1450.45	C-H bending, Alkane
9	1392	O-H bending, Phenol
10	1248.54	C-O Stretching, Alkyl aryl ether
11	1166	C-O Stretching, Aliphatic ether
12	607	C-I Stretching, halo compound
13	521	C-I Stretching, halo compound

Similar findings were reported in red algae *Gracilaria corticata* and *Gelidium pusillum* [20]. FTIR spectroscopy has become a more admired developed technique due to its easy sample preparation with a small sample size requirement. Most FTIR studies on the seaweed extracts revealed the interface of toxic sites, likely amino acid, carboxyl and hydroxyl groups. Like other natural molecules, algae show multifaceted vibrational spectra in almost all bands. The C-O stretching, NH stretching and O-H stretching bands are metal sensitive [21].

The results of the FTIR spectrum proved to be a presence of polyphenols with potential antioxidants properties [22, 23]. Several studies have shown that there are many types of research that exploit FTIR analysis for bioactive compounds [24, 25]. FTIR analysis for bioactive compounds [24, 25].

CONCLUSION: The phytochemical investigation of marine seaweed extract of *Champia compressa* suggested that it contains several important secondary metabolites like phenols, alkaloids, terpenoids and steroids, which contribute to its biological properties.

Hence, this methanolic seaweed extract was further implemented by separating, isolating, and identifying compounds and purification of a lead molecule by justifying its biological potentialities in future perspectives.

Authors' Contribution: The authors envisaged, intended, and analyzed the corresponding experiments and contributed to manuscript grounding.

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Ethical Approvals: The study does not involve any experiments on animals or humans.

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CONFLICT OF INTERESTS: The authors declare that they have no conflict of interest.

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