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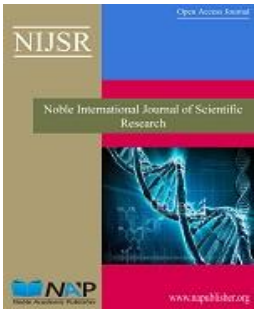

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Phytochemical Screenings of the Marine Red Alga, *Gracilaria Corticata*

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Abstract: The present study was aimed to understand the phytochemical screenings of the red alga, *Gracilaria corticata* was subjected to hexanic, acetonic and methanolic extactions. Hexanic extract of *G. corticata* showed presence of alkaloids, terpenoids, flavonoids, polyphenols and quinones. Acetonic extract of *G. corticata* showed presence of alkaloids, tannins, polyphenols, saponins, cardiac glycosides and quinones. In case, methanolic extract of *G. corticata* contained tannins, polyphenols, saponins, cardiac glycosides and quinones. Further, GC-MS analyses of *G. corticata* revealed that presence of 17 secondary compounds (6 from hexanic, 4 from acetonic and 7 from methanolic extracts). Among the 17 compounds, 8 compounds (1 from acetonic and 6 from methanolic and 1 from all the three extracts) are possessed bioactive properties based on literature. Thus, *G. corticata* has significant amount of primary and secondary phytochemicals. Among the three solvents extractions, methanolic extract possessed more numbers of secondary as well as bioactive compounds.

Keywords: Red Alga, Phytochemicals, Bioactive Compounds.

1. Introduction

Marine macro algae are renewable resource in marine environment, nearly 6000 species of marine algae have been identified, and they are classified into three different categories based on their pigmentation (chrophyta, phaeophya and rhodophyta). Macro algae are naturally renewable sources which are used as food, feed and fertilizer in throughout the world. The marine algae contain a significant amount of essential vital nutrients: the reactive antioxidant molecules such as ascorbate and glutathione as well as secondary metabolites including carotenoids (α- and β - carotene, fucoxanthin, astaxanthin), mycosporine-like amino acids (mycosporineglycine) and catechins (e.g. catechin, epigallocatechin), gallate, phlorotannins (e.g. phloroglucinol), eckol and tocopherols), protein, vitamins (A, B, B_{12}, C, D, E, riboflavin, niacin, panthothanic acid and folic acid) and minerals (Ca, P, Na, K, S, Mg, Fe etc.) [1].

Marine algae are able to biosynthesize secondary metabolites that can mediate a broad range of intra and inter specific ecological interactions between organisms including chemical defenses [2]. The components reported to be found are sterols (some are fucosterol), different molecules containing vinyl and ethyl cholesterol types, cyclohexane and some sulfated polysaccharides fucoidan, neutral glucan and guluronic and mannuronic acid residues containing alginic acid providing a medicinal value for the brown and red algae [3, 4]. There are numerous reports of compounds derived from macro algae with a broad range of biological activities, such as antibacterial, antiviral, antifungal, antitumoral and anticoagulant [5-8]. In this line, the present work was aimed to analysis of profiles of the primary and secondary phytochemicals of red alga, *G. corticata*.

2. Material and Methods

2.1. Collection and Identification

The marine red alga, *G. corticata* was collected from the intertidal region of Mandapam coast (Lat. 9° 17'N; Lon. 79° 19'E) of Gulf of Mannar, south-east coast of Tamil Nadu, India. This macro algal species was identified based on its morphology by using identification manual of "Economically

Important Seaweeds" [9] published by Central Marine Fisheries Research Institute (ICAR), Kochi, India. Finally, the species was authenticated by Botanical Survey of India (BSI), Coimbatore, India.

2.2. Solvent Extraction of *G. Corticata*

The collected sample was cleaned well with seawater to remove all the extraneous matter such as epiphytes, sand particles and necrotic parts and brought to the laboratory in plastic bags. The sample was then thoroughly washed with freshwater, blotted, spread out and dried at room temperature for 2 weeks. Shade dried sample was ground to fine powder. The powdered sample was stored in sterilized containers for further usage. The powdered sample of *G. corticata* (50 g) was packed in Whatmann No. 1 filter paper and Soxhlet extraction was done with 300 ml (1:6 w/v) of hexane, acetone and methanol individually for 6-9 h (30 to 36 cycle) until a clear colorless solution was obtained. Fresh *G. corticata* powder was used for each solvent extraction. These extract were filtered by using double layer muslin cloth, concentrated at 40-50 °C using rotary vacuum evaporator (ROTAVAP) attached with ultra-cryostat and dried at 40 °C under hot air oven [10]. The dark, gummy solid obtained were used for further investigation.

2.3. Extract Recovery Percentage

The extracts of *G. corticata* recovered after Soxhlet was calculated [11]:

Recovery % = Extract weight / Plant sample weight (g) × 100

2.4. Qualitative analysis of Phytochemicals

Each solvent extract was subjected to primary phytochemical analysis such as presence of alkaloids, terpenoids, flavonoids, tannins, polyphenols, saponins, cardiac glycosides and quinones by adopting the standard qualitative procedures [12].

2.5. Gas Chromatography-mass Spectrum (GC-MS) Analysis

Each extracts of *G. corticata* were subjected to GC-MS (The Trace GC Ultra and DSQII model MS with inbuilt pre-filter to reduce the neutral particles, Thermo Fisher Scientific Company Pvt. Ltd.) analysis for identification of different secondary phytochemical compounds with following working conditions [Injector port temperature: 250^{o}C; Interface temperature: 250^{o}C and source was maintained at 200^{o}C; The oven temperature: programmed as variable, 70^{o}C for 2 mins, 150^{o}C @ 8^{o}C /min, up to 260^{o}C @ 10^{o}C /min; and the injector was used splitless mode; Column: The DB-35 MS Nonpolar (Agilent Co., USA) with dimensions of 0.25 mm OD x 0.25 µm ID x 30 metres length; Carrier gas: Helium was used at 1 mL/min; Scan: 50-650 Da; Motor vacuum pressure: <40; Ionization energy: -70eV].

Peaks resolved with relative abundance of 0-100 were considered as major compounds. To show the minor peaks, the chromatogram was magnified. Identification of various components present in each extract was done by comparison of retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literature. National Institute Standard and Technology (NIST4) and WILEY9 [13] on-line library source were also used for matching the identified components.

3. Results and Discussion

3.1. Extraction Yield of *G. Corticata*

The successive solvent extract yield was shown in the figure 1. The compounds of *G. corticata* present in each solvent extract were according to non-polar to polar nature. In *G. corticata*, hexanic, acetonic and methanolic extracts yielded 0.20%, 0.39% and 1.4% respectively. The polar solvent, methanolic yield was the best, followed by the middle polar solvent, acetonic and nonpolar solvent hexanic extracts.

3.2. Primary Phytochemicals of *G. corticata*

The hexanic extract of *G. corticata* showed presence of 5 primary compounds such as alkaloids, terpenoids, flavonoids, polyphenols and quinones of which alkaloids was luxuriantly present. Terpenoids, polyphenols and quinones were moderately present. Acetonic extract of *G. corticata* showed presence of 6 compounds of which quinones was luxuriantly present. Tannins, polyphenols, saponins and cardiac glycosides were moderately present. The other compound such as alkaloids was poorly present. In case, methanolic extract of *G. corticata* contained 5 primary compounds such as tannins, polyphenols,

saponins, cardiac glycosides and quinones of which polyphenols, cardiac glycosides, saponins and quinones were luxuriantly present. Tannins was moderately present (Table 1). The algal extraction was mostly done with polar solvents. More yield was depending upon the solvent type which dissolves more of a particular compound. Hence, the methanolic extraction of *G. corticata* were contains more yields followed by other solvents.

Alkaloids are reported to be biologically and therapeutically active (e.g. morphine, atropine and quinine) and have numerous medical applications [14]. In the present study, alkaloids was luxuriantly present in hexanic extract of *G. corticata*, whereas, poorly present in acetonic extract of *G. corticata.*

Terpenoids are reported to be useful in the prevention and therapy of several diseases including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, anti-viral, anti-allergenic, anti-spasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties [15]. In the present study, terpenoids are moderately present in hexanic extract of *G. corticata.*

Flavonoids are reported to possess antioxidant, free radical scavenger, antileukemic, vasodilator and antibacterial properties and are reported to be useful for improving blood circulation in brain of Alzheimeric patients [16]. In the present study, moderately present in in hexanic extract of *G. corticata.* Tannins are used in medicine as mild antiseptics in treatment of diarrhea and to check small hemorrhages [17]. In the present study, acetonic and methanolic extracts were showed moderately presence of tannins in *G. corticata*.

Phenols are structural and allelopathic components which are associated with diverse functions including activation of enzymes, nutrient uptake, protein synthesis and photosynthesis [18]. In the present study, moderate to luxuriant presence of polyphenols was detected in all the three solvents extracts *G. corticata*.

Saponins have a wide range of medicinal properties including hypo-cholesterolemic, anticarcinogenic, anti-inflammatory, anti-microbial and antioxidant [19]. In the present study, moderate to luxuriant presence of saponins was seen in *G. corticata* extract of which, moderately present in acetonic and luxuriantly present in methanolic extracts of *G. corticata.*

The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal. Cardiac glycosides and catecchloamine are agents of choice in treatment of congestive cardiac failure [20]. In the present study, moderate present in acetonic and luxuriantly present in methanolic extracts of *G. corticata.* Quinones are compounds very much used in pharmacopoeia in the treatment of malaria [21] and more recently of tumours [22]. They are having good source of anti- inflammatory, antibacterial and immunomodulating potentials [23]. In the present study, in *G. corticata* moderate to luxuriant presence of quinones was detected in all the three solvents extracts of which hexanic extract showed moderate presence and acetonic and methanolic extracts showed luxuriant presence. The presence of alkaloids, flavonoids, phenols, saponins, glycosides, tannins, quinones, anthraquinones, catechin, steroids, sugar, amino acid, xanthoprotein and fixed oils are reported in *G. corticata* [24].

3.3. Secondary Phytoconstituents of *G. corticata*

GC-MS analyses of hexanic extract of *G. corticata* revealed that presence of 6 different secondary compounds {O-Toluic acid, tridec-2-ynyl ester; (5RS,6RS)-5-Hydroxy-6-phenylmethyl-2-piperidinone; N-Acetyl-a-(a'-naphthyl)-glycine; 3-[O,O-Diethyl phosphoryl] -5-iso-butoxy-2-(trifluoro methyl)-2,5-dihydrofuran; (E)-Dodec-5-en-4-olide; 13-Docosenamide, (Z)-**}**, of which 1 compound has bioactive properties, namely, 13-Docosenamide, (Z)- (Table 2; Fig. 2).

Acetonic extract of *G. corticata* showed presence of 4 different secondary compounds {3-Methylhexyl isothiocyanate; 8,9,10,11-Tetrahydrobeno [a]chinolizine-6,11-dione; Cholesterol; 13-Docosenamide, (Z)-} of which 2 compounds {3-Methylhexyl isothiocyanate; 13-Docosenamide, (Z)-} having biological properties (Table 3; Fig. 3).

In the case of methanolic extract of *G. corticata* it contains 7 different secondary compounds {2-[5-(2-Hydroxy-propyl)-tetrahydrofuran-2-yl]-propionic acid, t-butyl ester; heptadecane; neophytadiene; hexadecanoic acid; 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]- ; 5a-Cholestane-3a,25-diol; 13-Docosenamide, (Z)-} of which all the 7 compounds are having bioactive principles (Table 4; Fig. 4).

Only one bioactive compound, 13-Docosenamide, (Z)- was detected in all the three solvents extractions of *G. corticata.* Based on the literature [25-35], the identified secondary bioactive compounds present in *G. corticata* are presented in tables 2-4 and figures 2-4. Totally from all the three solvents extractions, 8 different bioactive compounds, 2-[5-(2-Hydroxy-propyl)-tetrahydrofuran-2-yl]-propionic

acid, t-butyl ester; heptadecane; neophytadiene; hexadecanoic acid; 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]-; 5a-Cholestane-3a,25-diol; 13-Docosenamide, (Z)-; {3-Methylhexyl isothiocyanate were detected in *G. corticata*.

4. Conclusion

The present study revealed that *G. corticata* contained significant amount of primary and secondary phytochemical constituents. Therefore, it is suggested that isolation, purification and characterization of individual bioactive compounds of *G. corticata* to study their unique pharmaceutical active principles.

Acknowledgement

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Table 1. The primary phytochemicals present in *G. corticata* extracted with different solvents.

Phytochemicals	Different solvents		
	Hexane (non-polar)	**Acetone (middle-polar)**	**Methanol (Polar)**
Alkaloids	+++	+	--
Terpenoids	++	--	--
Flavonoids	++	--	--
Tannins	--	+	++
Polyphenols	++	++	+++
Saponins	--	++	+++
Cardiac glycosides	--	++	+++
Quinones	++	+++	+++

+, Present; ++, Moderately present; +++, Luxuriantly present; --, Absent

Table 2. GC-MS profiles of secondary phytochemical compounds of *G. corticata* extracted with hexane.

RT	Name of the compounds	P	MF	MW	Area (%)	SI	RSI	Biological properties by literature only
10.48	NV	NV	NV	NV	NV	NV	NV	NV
14.51	O-Toluic acid, tridec-2-ynyl ester	29.97	$C_{21}H_{30}O_2$	314	9.91	541	728	--
18.66	(5RS,6RS)-5-Hydroxy-6-phenylmethyl-2-piperidinone	47.48	$C_{12}H_{15}O_2$	205	7.37	402	829	--
22.89	N-Acetyl-a-(a'-naphthyl)-glycine	51.68	$C_{14}H_{13}NO_3$	243	24.73	403	912	--
26.29	3-[O,O-Diethyl phosphoryl] -5-iso-butoxy-2-(trifluoro methyl)-2,5-dihydrofuran	26.13	$C_{13}H_{22}F_3O_5P$	346	42.14	380	798	--
30.46	(E)-Dodec-5-en-4-olide	21.24	$C_{12}H_{20}O_2$	196	0.40	617	706	--
35.34	13-Docosenamide, (Z)-	75.70	$C_{22}H_{43}NO$	337	1.20	845	854	Anti-inflammatory activity [25]

RT, Retention time; NV, Not validated; P, Probability; MF, Molecular formula; MW, Molecular weight; SI, Similar index; RSI, Reverse similar index

Table 3. GC-MS profiles of secondary phytochemical compounds of *G. corticata* extracted with acetone.

RT	Name of the compounds	P	MF	MW	Area (%)	SI	RSI	Biological properties by literature only
10.76	NV	--	--	--	--	--	--	--
14.25	3-Methylhexyl isothiocyanate	10.14	$C_8H_{15}NS$	157	2.84	377	517	Antibacterial activity [26]
22.54	8,9,10,11-Tetrahydrobeno [a]chinolizine-6,11-dione	16.00	$C_{13}H_{11}NO_2$	213	3.17	449	865	--
31.22	Cholesterol	39.30	$C_{27}H_{46}O$	386	73.64	912	914	--
35.33	13-Docosenamide, (Z)-	74.32	$C_{22}H_{43}NO$	337	3.38	802	825	Anti-inflammatory activity [25]

RT, Retention time; NV, Not validated; P, Probability; MF, Molecular formula; MW, Molecular weight; SI, Similar index; RSI, Reverse similar index

Table 4. GC-MS profiles of secondary phytochemical compounds of *G. corticata* extracted with methanol.

RT	Name of the compounds	P	MF	MW	Area (%)	SI	RSI	Biological properties by literature only
7.63	NV	--	--	--	--	--	--	--
14.41	2-[5-(2-Hydroxy-propyl)-tetrahydrofuran-2-yl]-propionic acid, t-butyl ester	12.48	$C_{14}H_{26}O_4$	258	1.33	371	447	Antioxidant, free radicals [27]
17.00	Heptadecane	34.97	$C_{17}H_{36}$	240	3.09	923	924	Antibacterial, antifungal, antiviral, antioxidant [28]
19.85	Neophytadiene	36.48	$C_{20}H_{38}$	278	1.66	888	907	Antibacterial, antipyretic, analgesic, anti-inflammatory, antimicrobial and antioxidant [29, 30]
22.48	Hexadecanoic acid	69.37	$C_{16}H_{32}O_2$	256	8.46	861	864	Anti-inflammatory, antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, haemolytic, 5-alpha reductase inhibitor, mosquito larvicide [31-33]
25.12	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]-	67.27	$C_{20}H_{40}O$	296	3.32	891	936	Antimicrobial, anticancer, anti-inflammatory, antidiuretic, immunostimulatory and anti-diabetic [34]
30.94	5a-Cholestane-3a,25-diol	60.79	$C_{27}H_{48}O_2$	404	29.44	812	894	Anticancer activity, antitumor, antimicrobial [35]
35.34	13-Docosenamide, (Z)-	67.26	$C_{22}H_{43}NO$	337	12.42	813	825	Anti-inflammatory activity [25]

RT, Retention time; NV, Not validated; P, Probability; MF, Molecular formula; MW, Molecular weight; SI, Similar index; RSI, Reverse similar index.

Fig. 1. Percentage yield of total phytochemicals in different solvents extracts of *G. corticata*.

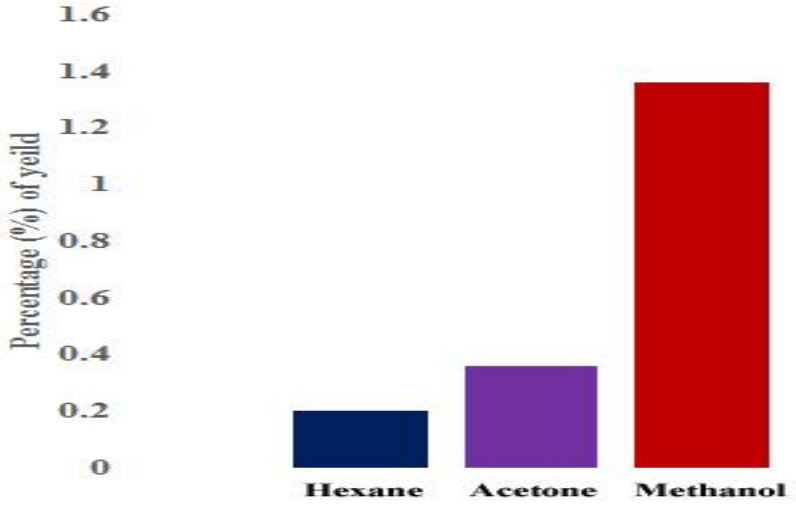

Fig. 2. GC- MS chromatogram of *G. corticata* extracted by using hexane

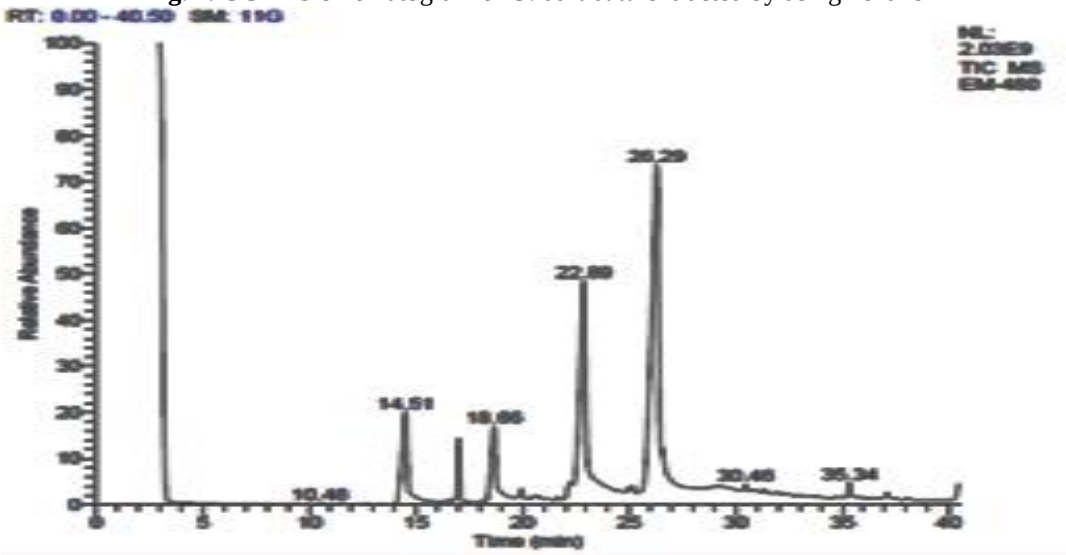

Fig. 3. GC- MS chromatogram of *G. corticata* extracted by using acetone.

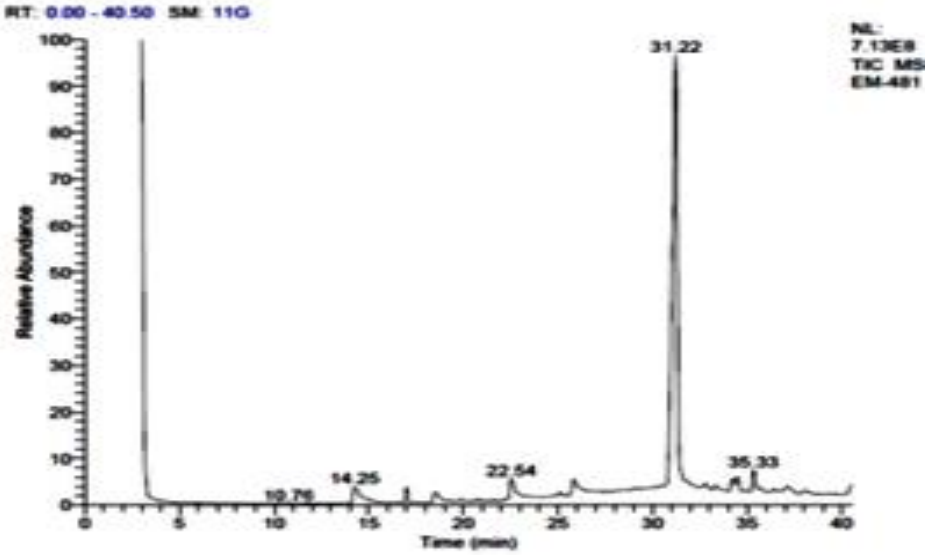

Fig. 4. GC- MS chromatogram of *G. corticata* extracted by using methanol