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Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 03

Res Jr of Agril Sci (2021) 12: 894–898

CARAS

Res. Jr. of Agril. Sci. (May-Jun 2021) 12(3): 894–898
www.rjas.org

Full Length Research Article

Qualitative and Quantitative Screening of Phytochemicals in Selected Brown Seaweed (*Turbinaria ornata*) Collected from Gulf of Mannar, Tamil Nadu, India

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Received: 29 Mar 2021 | Revised accepted: 16 May 2021 | Published online: 25 May 2021

A B S T R A C T

Turbinaria ornata is a species of marine brown seaweed and in the family Sargassaceae. They were freshly collected from Mandapam Coastal Area, Tamil Nadu, India and rinsed in seawater and packed in aseptic bags for further proceedings to laboratory. Seaweeds are potential renewable resources in the marine environment. It has been used as antioxidant and antimutagen. Hence, the present study was carried out to exhibit the preliminary phytochemical screening and invitro antioxidant properties of various extracts of *Turbinaria Ornata*. Methanol, ethanol, and water extracts were prepared for further analysis. Out of three extracts, ethanolic extract showed the maximum phytoconstituents. The results of the present study revealed the presence of tannin, saponin, flavonoids, steroids, terpenoids, alkaloids, phenols, anthroquinone, glycosides and coumarins. Our study also demonstrated the good invitro antioxidant efficacy of ethanolic extract of *Turbinaria ornata*. Therefore, we concluded that *Turbinaria ornata* may be used as a rich source of phytoconstituents and natural antioxidants.

***Key words*:** Brown seaweed, Phytochemical screening, Invitro antioxidant, *Turbinaria ornata,* Efficacy

Seaweeds are possible renewable sources in the marine natural environment. It is generated immense volume of bioactive compounds with enormous medicinal potential. Now, the aids of antibiotics have risen owing to infections. The initial inquiry of antibiotic activity borne out by Pratt *et al*. [1]. Since algae have been used in conventional medicine for a long time. Seaweed is rich in antioxidants such as carotenoids, pigments, polyphenols, enzymes [1]. Most of the seaweed we consume is as food, feed, and fertilizer in many parts of the world. They constitute a vital part of marine ecosystems. These seaweeds are reservoirs of pigments, polyphenols, enzymes, carotenoids, diverse functional polysaccharides [2-3]. Amongst the distinct compounds with functional properties, antioxidants are the most extensively investigated. Antioxidants are the substances, which can secure serious human disorders including melanoma, cardiac syndromes, diabetes, cancer, inflammatory that describe their potential use in expanding shelf life of food and as medicine [4]. *Turbinaria ornata* (Turner) is an extensive species of marine green alga belonging to the family Phaeophyceae and is rich in fucoids and sulphated polysaccharides [5]. Brown macroalgae species are widely studied for their potential pharmaceutical use. They are found to have antioxidant, antidiabetic, anti-inflammatory, antiviral, antiproliferative, and anticoagulant properties [6]. While thousands of bioactive compounds have been exposed, the need for novel therapeutic compounds is still vital in concern of number of new diseases and resistant strains of microorganisms. Hence, the present study was carried out to screening of phytochemicals and invitro antioxidant properties of various extracts of *Turbinaria ornata*.

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MATERIALS AND METHODS

Collection of seaweeds

Turbinaria ornata were collected from Gulf of Mannar, Rameswaram, Tamil Nadu, India. The collected samples were cleaned well with sea water to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in sterile bags. Then the samples were washed with tap water and distilled water and spread in the dark room for drying, after which the dried samples were powdered and subsequently stored at 4°C.

Preparation of extract

10gms of the powder of *Turbinaria ornata* were transferred into three different conical flasks (250ml). The conical flask containing 100ml of three different solvents viz. Ethanol, Methanol, and water. The conical flask containing *Turbinaria ornata* powder and solvent was shaked it well for

30 minutes by free hand. After 24 hours, the extracts were filtered using Whatman filter paper No.1. and filtrate used for further analysis. The obtained extracts were stored at 4°C in airtight bottle until further use.

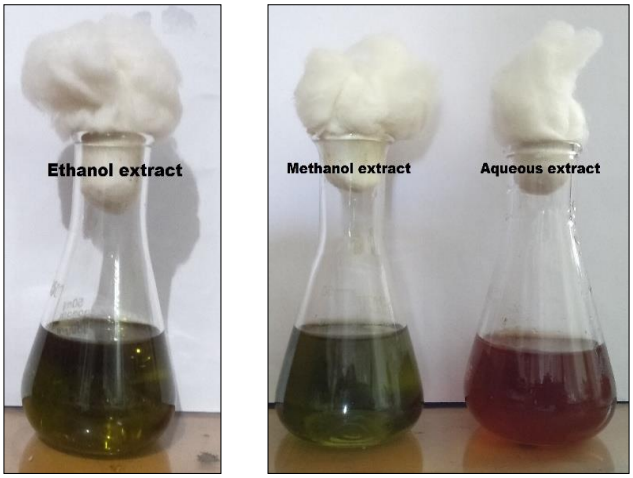

Fig 1 Preparation of extract

Qualitative preliminary phytochemicals screening

Chemical tests were carried out on the various of *Turbinaria ornata* extract using standard procedures to identify the phytochemicals namely Anthrauinones, Alkaloids, Carbohydrates, Aminoacids, Polyphenols, Tannins, Phlobatannins, Saponins, Flavonoids, Terpenoids, Triterpenoids, Glycosides following the methodology of Sofowara [7], Trease and Evans [8] and Harborne [9].

Quantitative analysis of phytochemicals

Determination of total phenols by spectrophotometric method

Total phenols were estimated by the method of Edeoga *et al*. [10].

Determination of total flavonoids

The amount of flavonoids were determined by the method of Bohm and Kocipai-Abyazan [11].

Determination of tannin

The amount of tannin was determined by the method of Van-Burden and Robinson [12].

Determination of saponin

The amount of saponins was determined by the method of Obadoni and Ochuko [13].

Determination of alkaloid

The amount of alkaloid were estimated by the method of Harborne [14].

In vitro anti-oxidant activity of Turbinaria ornata

Preparation of extract

Different concentrations of *Turbinaria ornata* **(**20, 40, 60 and 80 µg/ml) were chosen for *in vitro* antioxidant activity. L-Ascorbic acid was used as the standard.

DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by the method of Shimada *et al.* [15].

Determination of total antioxidant capacity

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*. [16].

Superoxide anion scavenging activity assay

The superoxide anion radicals scavenging activity was measured by the method of Liu *et al*. [17].

Nitric oxide scavenging activity assay

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat [18].

Reducing power assay

The Fe^{3+} reducing power of the extract was determined by the method of Oyaizu [19].

Statistical analysis

Tests were carried out in triplicate for 3 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically determined by a linear regression method using Ms- Windows based graphpad Instat (version 3) software. Results were expressed as graphically / mean ± standard deviation.

RESULTS AND DISCUSSION

For the pharmacological study of novel drugs, the essential information regarding the chemical constituents is generally provided by the qualitative phytochemical screening of plant extracts. The qualitative tests of extracts showed significant indication about the presence of metabolites. Standardization is an essential measurement for ensuring the quality control of the herbal.

The appearance and percent extract ability of all the extracts are shown in (Table 1). The results of preliminary phytochemical screening revealed the presence of carbohydrates, tannins, glycosides, flavonoid, phenolics, saponins and volatile oils. The result of preliminary phytochemical screening of various extracts revealed the presence of various phytoconstituents, as shown in (Table 1). The result showed the presence of more amount of the above-mentioned phytochemicals in ethanolic extract as compared to aqueous and methanolic extract as shown in (Fig 2a-c).

Table 1 Qualitative analysis of different extract of *Turninaria ornata*

Phytochemicals	Ethanolic extract	Methanolic extract	Aqueous extract
Tannin	+	+	+
Saponin	++	+	+
Flavonoids	++	+	+
Steroids	++	+	+
Terpenoids	++	+	+
Triterpenoids	+	+	+
Alkaloids	+	+	-
Antroquinone	+	+	+
Polyphenol	++	++	+
Glycoside	+	+	+
Coumarins	+	+	+
Emodins	-	-	-
Anthocyanins	-	-	-

(-) Indicates Absence; (+) Indicates Presence; (++) High intensity (concentration) of colour

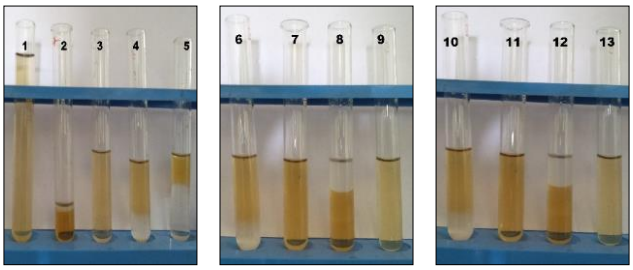

Fig 2a- Ethanolic extract of *Turbinaria ornata*

Table 2 Quantitative analysis of ethanolic extract of *Turbinaria ornata*

Phytochemicals	Results (mg/gm)
Flavonoids	136.48±9.55
Tannin	68.14±4.76
Phenol	182.56±12.74
Saponin	104.32±7.30
Alkaloids	52.32±3.66

Value expressed as Mean ± SD for triplicates

Total phenolic, flavonoid, tannins, saponin and alkaloid contents of all the extracts were determined by UV spectrophotometric method. The total phenolic and flavonoid contents were found in *Turbinaria ornata* 182.56±12.74 and 136.48±9.55 mg/g, respectively results shown in (Table 2). The given values are mean ± SD of three different determinations. The tannin, saponin and alkaloids contents were found in *Turbinaria ornata* 68.14±4.76, 104.32±7.30 and 52.32±3.66 mg/g, respectively results shown in (Table 2).

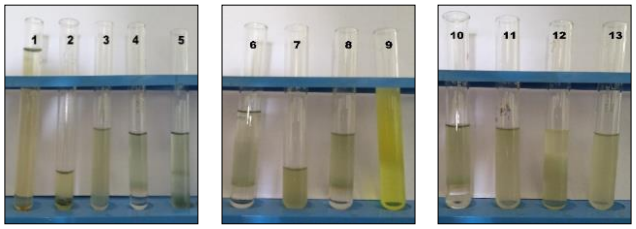

Fig 2b- Methanolic extract of *Turbinaria ornata*

Fig 2c- Aqueous extract of *Turbinaria ornata*

1. Tannin, 2. Saponnin, 3. Flavonoids, 4. Steroids, 5. Terpenoids, 6. Triterpenoids, 7. Alkaloids, 8. Anthroquinone, 9. Polyphenol, 10. Glycoside, 11. Coumarins, 12. Emodins and 13. Anthocyanin

Table 3 DPPH radical scavenging activity of ethanolic extract and ascorbic acid at different concentrations

Concentration	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	IC_{50}
Ethanolic extract	23.18±1.62	46.81±3.27	63.18±4.42	84.54±5.91	45.58
Ascorbic acid (Std.)	25.90±1.81	51.81±3.62	72.27±5.05	96.81±6.77	39.96

Value expressed as Mean ± SD for triplicates

Table 4 Total antioxidant activity of ethanolic extract and ascorbic acid at different concentrations

Concentration	20 µg/ml	40µg/ml	60µg/ml	80µg/ml	IC_{50}
Ethanolic extract	22.81±1.59	45.93±3.21	61.56±4.30	83.43±5.84	46.52
Ascorbic acid (Std.)	25.31±1.77	48.12±3.36	69.37±4.85	92.81±6.49	42.04

Value expressed as Mean ± SD for triplicates

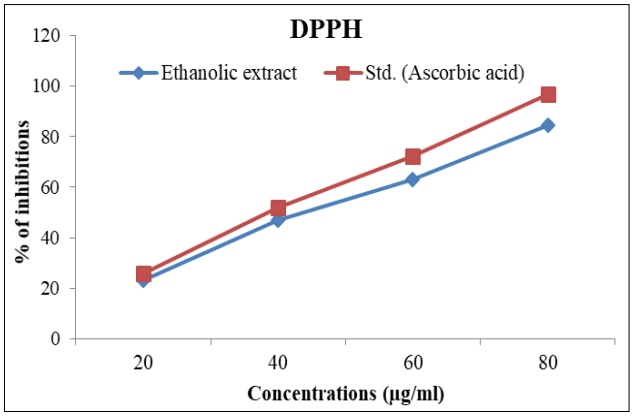

Fig 3 Percent of DPPH radical scavenging activity of ethanolic extract of *Turbinaria ornata* and ascorbic acid at different concentrations

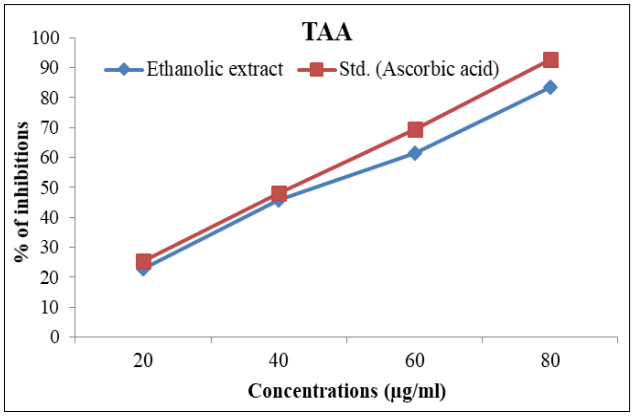

Fig 4 Percent of total antioxidant activity of ethanolic extract of *Turbinaria ornata* and ascorbic acid at different concentrations

Table 5 Superoxide anion radical scavenging activity of ethanolic extract and ascorbic acid at different concentrations

Concentration	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	IC_{50}
Ethanolic extract	18.21±1.27	36.42±2.54	67.50±4.72	82.14±5.74	49.03
Ascorbic acid (Std.)	23.21±1.62	49.64±3.47	71.07±4.97	88.92±6.22	42.48

Value expressed as Mean ± SD for triplicates

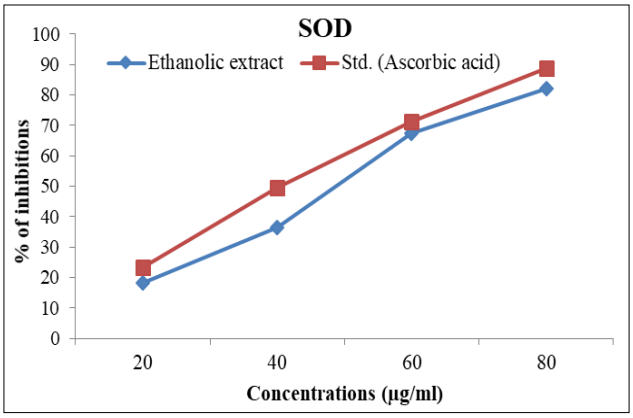

Fig 5 Percent of Superoxide anion radical scavenging activity of ethanolic extract of *Turbinaria ornata* and ascorbic acid at different concentrations

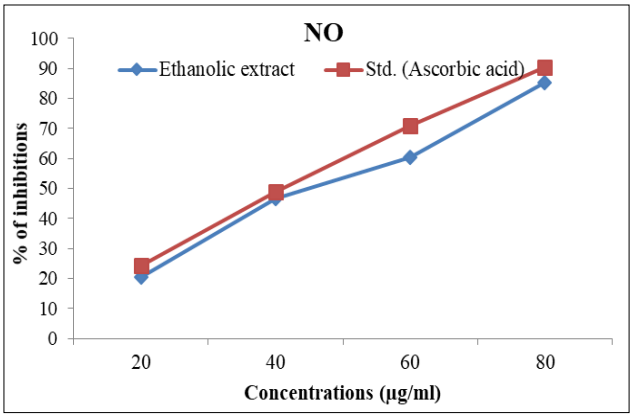

Fig 6 Percent of Nitric oxide scavenging activity of ethanolic extract of *Turbinaria ornata* and ascorbic acid at different concentrations

Table 6 Nitric oxide scavenging activity of ethanolic extract and ascorbic acid at different concentrations

Concentration	20 µg/ml	40µg/ml	60µg/ml	80µg/ml	IC_{50}
Ethanolic extract	20.47±1.43	46.67±3.26	60.47±4.23	85.23±5.82	46.91
Ascorbic acid (Std.)	24.28±1.69	49.04±3.43	70.95±4.96	90.47±6.33	42.12

Value expressed as Mean ± SD for triplicates

Table 7 Reducing power activity of Ethanolic extract and ascorbic acid at different concentrations

Concentration	20 µg/ml	40µg/ml	60µg/ml	80µg/ml
Ethanolic extract	0.083±0.005	0.121±0.008	0.196±0.013	0.215±0.015
Ascorbic acid (Std.)	0.096±0.006	0.148±0.010	0.201±0.014	0.239±0.016

Value expressed as Mean ± SD for triplicates

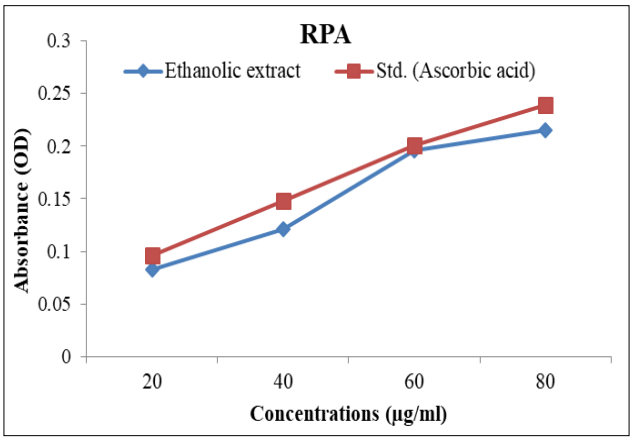

Fig 7 Percent of reducing power activity of ethanolic extract *Turbinaria ornata* and ascorbic acid at different concentrations

The present investigation revealed that the *Turbinaria ornata* extracts contain significant amount of phenols, flavonoids, tannins, saponins and alkaloids. The outcome of these findings might be useful as a diagnostic tool for the evaluation of these anticancer activity of seaweed.

CONCLUSION

Seaweeds collected from the Mandapam coast of India have been shown to possess several biological activities. In our studies, *Turbinaria ornata* were collected and examined for their qualitative and quantitative phytochemical constituents.

Acknowledgement

The authors are thankful to Harmann Research Institute, Thanjavur for their support to complete a part of analysis.

Conflicts of interest: There are no conflicts of interest.

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