Preliminary study on phytonutrients constituents and the anti-oxidant potential of *Gracilaria opuntia* of Rameswaram Coast, India.

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Abstract
Objective: Preliminary phytonutrient and invitro evaluation of *Gracilaria opuntia.*
Methods: Phytonutrient evaluation studied with various extracts like ethyl acetate, ethanol and purified water. The antioxidant potential was studied by DPPH, H_2O_2, NO and OH^- assays.
Results: The phytonutrient screening studies exhibits existence of various nutrient substituents. Also, a comprehensive anti-oxidant potential at 500 µg/ml in *Gracilaria opuntia* comparatively better than used standard.
Conclusion: Thus *Gracilaria opuntia* can be consider for curative disease from oxidative weakening in which *in-vivo* studies can be evaluated in advance.

Keywords: Gracilaria, Seaweed, Phytonutrient, Polyphenol compounds, Antioxidant.

INTRODUCTION

Natural ways stand as a golden mark to exemplify the outstanding phenomena of symbiosis. In India plants and herbal products in practice for health and medicinal purpose for several thousand years, there are nearly 1.5 million practitioners till using traditional medicine system such as Ayurveda, Siddha, Unani and Homeopathy. (Sheeta Verma *et al., 2008*). The main benefits of herbal medicines over allopathic are that it has least side effect, toxicity and cozy effective (Patil S Jolly *et al.*, 2003, Anggadireja J *et al.*, 1997, Bors W *et al.*, 1990). Antioxidants is a substance that is existing at low concentrations at which notably delays or inhibits oxidation of substrates. (Bhuvaneswar *et al*.,2013) They are preferred defense against free radical damage and maintain finest health for well -being. (Vishnu Kiran *2014*, Velavan S *2011*, Halliwell B *et al.*,

1997, Ramdani M *et al.,* 2017). The main background of the research work is to evaluate the *in vitro* antioxidant activity of Gracilaria opuntia of various extracts based on the comparison with standard.

MATERIALS & METHODS:

Collection of Algae Sample:

Gracilaria opuntia was collected from R.K. Algae project centre, Rameswaram Coast, India. The seaweed was authenticated under Botanical Survey of India, Calcutta.

Preparation of Seaweed Extracts:

Gracilaria opuntia seaweed saturated in running water and dried, powdered weighed accurately in the ratio of 1:3, accordingly volumes of ethyl acetate, ethanol and distilled water separately extraction process conducted. (Gajalakshmi *et al.,* 2018). The ethyl acetate, ethanol and aqueous extracts were freeze-dried, and kept in air-tight plastic containers. (Eun-sun Hwang *et al., 2014)*

Phytonutrient screening

Phytonutrient analysis for the test of alkaloids, tannins, flavonoids, steroids, terpenoids, triterpenoids, anthraquinones, polyphenols, proteins, glycoside and carbohydrates of all the extracts was conducted as per Indian pharmacopoeia. (O' Sullivan AM *et al.,* 2011)

In Vitro Antioxidant Studies

DPPH (1,1-DI Phenyl-2-Picryl Hydrazyl) Scavenging Activity:

To find free radical scavenging activity, test samples (0.5ml seaweed sample and standard amino acid) of different concentrations (100-500µg/ml), (Qi H Zheo *et al.,* 2005, Ciz M *et al.,* 2010) and 0.3 ml of DPPH solution (0.5 mM dissolved in ethanol solvent) were prepared. Control which contains only 3.5 ml of ethanol and 0.3 ml DPPH solution. After 100 minutes, absorbance was measured at 517 nm. (Ganesan P *et al.,2008*).

Hydrogen Peroxide Scavenging Activity Test:

100-500µg/ml of test seaweed samples and standard ascorbic acid were prepared by dissolving in suitable solvent (Duh PD *et al.,* 1998) to each add 3.4 ml of phosphate buffer (pH- 7.4) along with 0.6 ml of prepared 40nM hydrogen peroxide solution, incubate at 37^0C for 5 minutes and absorbance was recorded at 230nm. (Lim SN *et al.,*2002, Darcy Vrillon B 1993)

Hydroxyl radical scavenging activity:

500µL of seaweed sample each (100-500µg/ml) mixed with 200 µL 1.04nM EDTA and 200 µL $FeCL_3$ (1:1 v/v), 100 µL of H_2O_2 (1.0mM) and 100 µL ascorbic acid as standard, incubated at 37ºC for 1 h. After mixture treated with 1.0mL of thiobarbituraic acid (1%) and 1.0 mL of trichloroacetic acid (2.8%) incubated at 100ºC for 20min. after cooling, absorbance is measured at 532 nm, against a blank sample. (Becka EM *et al.,* 2004)

Nitric oxide scavenging activity

0.5 mL of seaweed extract at various concentration (100-500µg/ml) with 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) incubated at 25ºC. (Frankel *et al.,* 2008) 0.5 mL of the incubated solution is mixed with 0.5 mL of Griess reagent after 2hrs of incubation at room temperature for 30min and absorbance measured at 546 nm. (Martinez Tome M *et al.,* 2001, Takamatsu S *et al.,* 2003)

STATISTICAL ANALYSIS

All the Experimental findings were assessed by using linear regression statistical analysis and the IC50 was calculated by plotting the graph as concentration versus percentage inhibition using MS Excel version MS OFFICE 2021.

RESULTS AND DISCUSSION

Phytonutrient screening studies has found that very high concentration of Proteins, steroids, phenols and high concentration of tannins, flavonoids, terpenoids, alkaloids, anthroquinones and reducing sugars were presence in ethyl acetate and ethanol extracts and in aqueous extract where very few phytonutrient constituents at low concentration found shown in table 1.

In vitro antioxidant studies

DPPH scavenging activity:

Activity of all three extracts and ascorbic acid as standard on DPPH scavenging activity at

various concentration (100-500 µg/ml) exhibited better percentage of inhibition comparable to standard at same concentration respectively. Maximum DPPH scavenging activity of ethyl acetate (500 µg/ml) showed highest inhibitory effect when compared to ethanol and aqueous extracts of seaweed shown in figure1. The IC_{50} value also better to the standard comparison shown in table 2.

Hydrogen Peroxide assay:
The highest inhibitory activity in all three seaweed extracts showed at 500 µg/ml and IC_{50} value shown in table 3. Ethyl acetate extract and ethanol extract shows better hydrogen peroxide scavenging activity than aqueous extract shown in figure 2.

Hydroxyl radical scavenging activity:
The effect of Hydroxyl radical was found and results shown as percentage of inhibition rate in figure 3. Ethyl acetate extract (500 µg/ml) exhibited the inhibition of about 86.27 is slightly better than standard shown 78.63 at same concentration respectively shown in table 4.

Nitric Oxide scavenging assay:
Nitric oxide scavenging activity on all three-seaweed extract shown in figure 4. The results show that ethyl acetate 69.52, ethanol extract shows 53.33 and aqueous extract 31.32 respectively. The IC_{50} value also shows ethyl acetate seaweed extract having relatively good compared to standard were shown in table 5.

DISCUSSION

In preliminary phytonutrient analysis ethyl acetate extract of *Gracilaria opuntia* exhibits superior contrast to ethanol and distilled water extracts. In DPPH scavenging activity and Hydrogen Peroxide assay 500µg/ml ethyl acetate extract exhibited better with highest inhibitory effect then other two extracts and standard. The Hydroxyl assay activity of ethyl acetate and ethanol extracts expressed almost similar in inhibitory effect with respect to standard. At the same time Nitric oxide antioxidant activity of *Gracilaria opuntia* ethyl acetate extract is superior at the concentration of 500 µg/ml. The IC_{50} comparatively better when compared to standard used.

CONCLUSION

The study on phytonutrient analysis of *Gracilaria opuntia* extract of ethyl acetate, ethanol and aqueous solvents having the beneficial secondary metabolites in seaweed. Thus, *Gracilaria opuntia* shows an abundant source of structurally novel and biochemical active metabolites compounds. From the results it was concluded that among three seaweed extracts ethyl acetate shows good antioxidant potential when compared to standard ascorbic acid in *in vitro* assay. The better antioxidant potential of *Gracilaria opuntia* extract have it made due to phenolic compounds presence. Thus, studied *Gracilaria opuntia* can be used as potential source of healthy food in diets with potential health effects like antioxidative.

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Table 1. Phytochemical constituents of Gracilaria opuntia

Phytochemical Test	EA Extract	ETOL Extract	DW Extract
Alkaloids	++	++	+
Tannins	++	+	+
Saponins	+	+	+
Steroids	+++	++	-
Terpenoids	++	+	-
Triterpenoids	+	+	-
Flavonoids	++	++	+
Reduced sugars	++	++	+
Anthraquinones	++	++	-
Proteins	+++	++	+
Polyphenols	+	+	-
Glycoside	+	+	-
Carbohydrates	++	++	+

Table 2. Effect of EA, ETOL, DW extracts of Gracilaria opuntia of DPPH Scavenging activity.

S.NO	Concentration (µg/ml)	Ascorbic acid (STD)	Ethyl Acetate	Ethanol	Aqueous
1	100	29.86	27.08	27	6.89
2	200	32.25	35.58	30.22	13.28
3	300	38.46	42.67	35.49	16.17
4	400	46.22	54.30	45.5	18.73
5	500	58.42	62.99	51.2	23.36
IC_{50}		444.06	359.84	495.58	1193.51

Table 3. Effect of EA, ETOL, DW extracts of Gracilaria opuntia of Hydrogen Peroxide Scavenging activity.

S.NO	Concentration (µg/ml)	Ascorbic acid (STD)	Ethyl Acetate	Ethanol	Aqueous
1	100	18.09	11.45	13.28	2.4
2	200	22.46	18.28	18.14	3.02
3	300	28.99	25.26	20.29	8.21
4	400	34.14	32.06	21.25	10.12
5	500	38.26	41.89	29.78	12.19
IC_{50}		715.00	872.71	1037.97	2016.84

Table 4. Effect of EA, ETOL, DW extracts of Gracilaria opuntia of Hydroxyl Scavenging activity.

S.NO	Concentration (µg/ml)	Ascorbic acid (STD)	Ethyl Acetate	Ethanol	Aqueous
1	100	26.43	42.17	31.84	9.43
2	200	32.31	49.17	42.31	16.17
3	300	56.45	62.13	51.45	23.37
4	400	64.83	74.03	63.15	30.22
5	500	78.63	86.27	71.25	41.05
IC_{50}		277.66	187.12	279.81	653.69

Table 5. Effect of EA, ETOL, DW extracts of Gracilaria opuntia of Nitric Oxide Scavenging activity.

S.NO	Concentration (µg/ml)	Ascorbic acid (STD)	Ethyl Acetate	Ethanol	Aqueous
1	100	17.83	19.43	17.13	7.02
2	200	30.21	31.24	24.12	11.07
3	300	49.62	46.74	33.14	17.05
4	400	61.42	57.34	41.32	22.31

5	500	81.23	69.52	53.33	31.28
IC_{50}		312.28	342.83	480.71	843.77

Figure 1. Percentage of inhibition of EA, ETOL, DW extracts of Gracilaria opuntia of DPPH Scavenging activity.

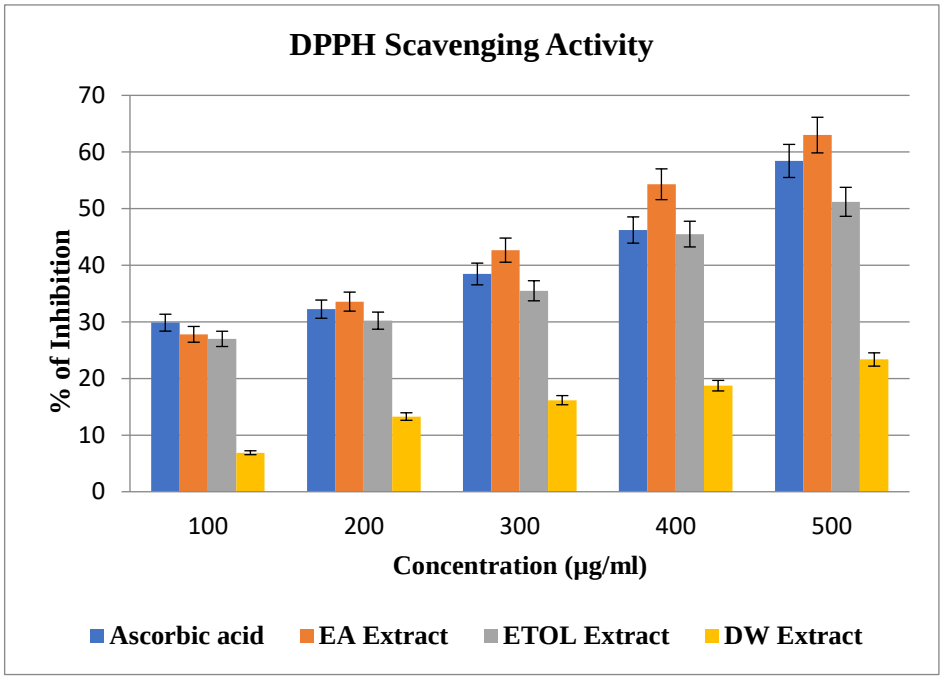


Figure 2. Percentage of inhibition of EA, ETOL, DW extracts of Gracilaria opuntia of Hydrogen Peroxide Scavenging activity.

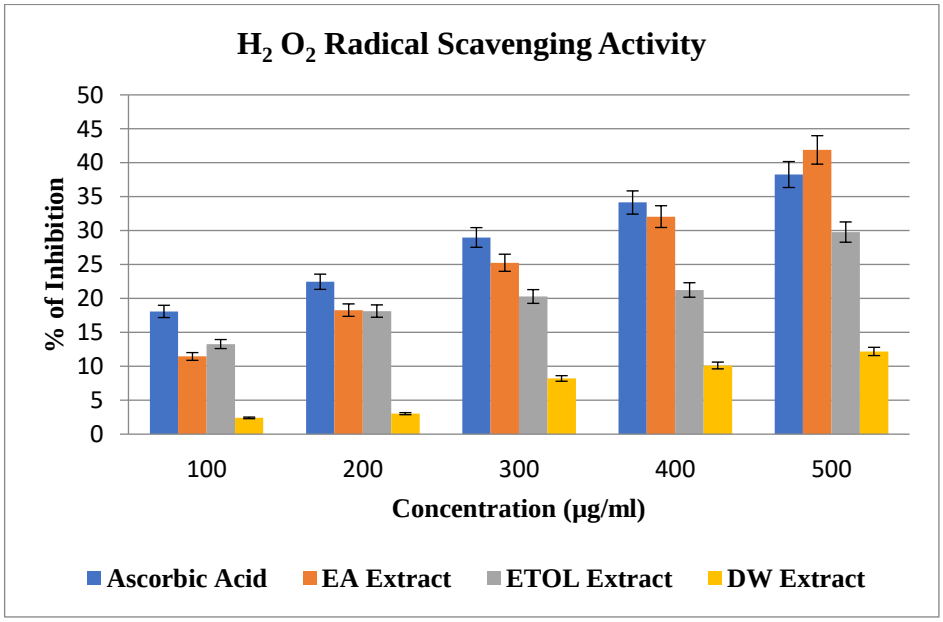


Figure 3. Percentage of inhibition of EA, ETOL, DW extracts of Gracilaria opuntia of Hydroxyl Scavenging activity.

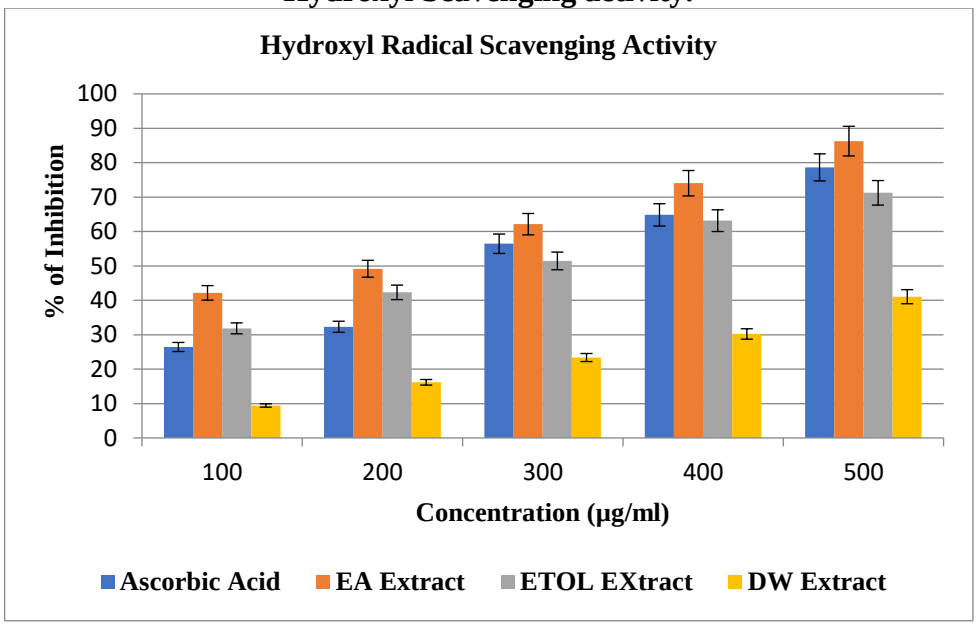
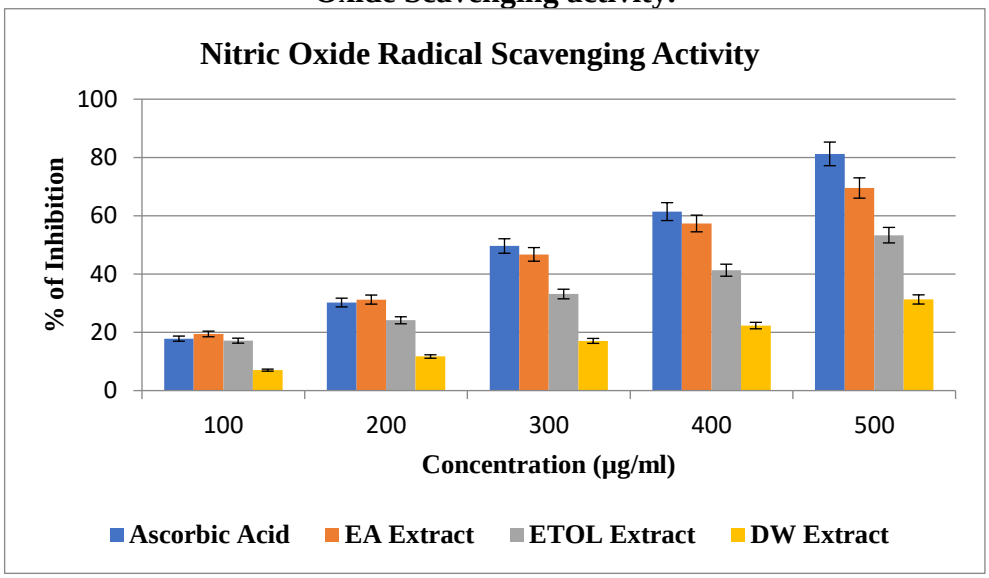


Figure 4. Percentage of inhibition of EA, ETOL, DW extracts of Gracilaria opuntia of Nitric Oxide Scavenging activity.



LIST OF ABBREVATIONS

S.NO	CODE	ABBREVATION
1.	ABR	Absorbance
2.	STD	Standard
3.	H2O2	Hydrogen peroxide
4.	DPPH	1,1-Diphenyl-2-picryl hydrazyl
5.	OH	Hydroxyl
6.	DNA	Deoxyribo nucleic acid
7.	ROS	Reactive oxygen species
8.	O2	Oxygen
9.	NOS	Nitric oxide synthase
10.	SOD	Super oxide dismutase
11.	BHT	Butylated hydroxyl toluene