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Original Article

EVALUATION OF *IN VITRO* ANTI-OXIDANT AND ANTI-ARTHRITIC ACTIVITY OF METHANOLIC EXTRACT OF MARINE GREEN ALGAE CAULERPA RACEMOSA

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ABSTRACT

Objective: The present study was aimed to evaluate *in vitro* anti-oxidant activity and *in vitro* anti arthritic activity of methanolic extract of *Caulerpa racemosa.*

Methods: The *in vitro* anti oxidant activity was evaluated by using following method viz; DPPH scavenging activity, Nitric oxide scavenging activity, Total anti-oxidant activity, Determination of reducing power. The *in vitro* anti arthritic activity was evaluated by using protein denaturation method.

Results: Methanolic extract of *Caulerpa racemosa* showed a very good anti-radical activity in scavenging DPPH radical and nitric oxide radical with maximum % inhibition of 88.87±1.05% at 2000µg/0.1 ml concentration and 80.49±1.43% at 2000µg/ml respectively. Total anti-oxidant activity and reducing power of *Caulerpa racemosa* was found to be 32µg equivalents of ascorbic acid at 500 µg/ml and 88.80±0.98% at 2000µg/0.05 ml respectively. The methanolic extract showed 49.33±0.597% of percentage inhibition at 1000µg/0.05 ml by protein denaturation method.

Conclusion: From the results obtained, it can be concluded that the methanolic extract of *Caulerpa racemosa* possesses significant anti-oxidant and anti-arthritic activity. Further studies are required to signify the mechanism of action of these pharmacological activities and to mark them out for their significant pharmacological actions.

Keywords: *Caulerpa racemosa,* Anti-oxidant, Diclofenac sodium, Anti-arthritic activity, Protein denaturation.

INTRODUCTION

Anti-oxidant is a molecule that inhibits oxidation of other molecules which produce free radicals. These radicals in turn produce chain reactions thereby cause damage to the cells, resulting in development of various ailments [1]. Anti-oxidants terminate these chain reactions by removing free radicals and inhibiting oxidative reactions. Therefore, antioxidants with free radical scavenging effect will be of greater importance in the prevention and therapeutics of the disease. Rheumatoid arthritis is a chronic inflammatory auto immune disease that exerts its greatest impact on the joints of the body that is lined with synovium, a specialized tissue responsible for maintaining the nutrition and lubrication of the joint [2, 3]. It is characterized by multiple conditions that cause pain, swelling, stiffness and loss of function in joints [4].

Caulerpa racemosa, marine green algae is a pan-tropical to temperate warm water species widely distributed throughout the world, found in many areas of shallow sea [5]. It consists of a number of branches linked to stolons of more than, with up to 200 fronds, which are anchored to sandy substrate by rhizoids and branches grown up to a height of 30 cm. Many spherical or ovate side shoots branch off and gives the seaweed its name 'bunch of grapes'. *Caulerpa racemosa* is used to lower blood pressure and to treat rheumatism [5]. It also contains compounds which function as a mild anesthetic, which gives the seaweed clinical value. On photochemical screening of methanolic extract, revealed the presence of various pharmacologically active compounds like steroids, triterpenoids, alkaloids, tannins, glycosides, carbohydrates, flavonoids and proteins. Therefore, the study was carried out to investigate the anti-oxidant and anti-arthritic activity of methanolic extract of *Caulerpa racemosa.*

MATERIALS AND METHODS

Chemical and reagents

Chemicals used in the studies were sodium nitroprusside, quercetin, Griess reagent, 1% sulphanilamide, 2% phosphoric acid, 0.1% napthyl ethylene diamineHcl, 1,1,-dipHenyl-2-picryl hydrazine (DPPH), dimethylsulphoxide (DMSO), potassium ferricyanide, trichloro acetic acid (TCA), ferric chloride, ascorbic acid, sulphuric acid, sodium phosphate, ammonium molybdate, bovine serum albumin, diclofenac sodium, 1N hydrochloric acid, methanol, phosphate buffer saline (PBS).

Plant material

Collection and authentication of plant materials

Caulerpa racemosa is a green marine algae of the family Caulerpaceae, which spread from tropical to warm water regions, was collected from Mandapam of Rameshwaram, Tamil Nadu in the month of October 2011. The plant was identified and authenticated by Dr. V. Krishnamurthy, Director, Krishnamurthy Institute of Algalogy, Chennai.

Preparation of extract

The *Caulerpa racemosa* was washed with sea water and then with tap water to remove the epiphytes and were shade dried, macerated with methanol exhaustively. The crude extract obtained after distillation of solvent at a lower temperature was used for the study.

Preliminary phytochemical screening

A portion of methanolic extract of *Caulerpa racemosa* was analyzed for the presence of various phytoconstituents [6]. Photochemical analysis exhibited the presence of steroids, triterpenoids, alkaloids, tannins, glycosides, carbohydrates, flavonoids and proteins.

In-vitro anti-oxidant activity

DPPH scavenging activity

DPPH scavenging activity was measured by the spectrophotometric method. A stock solution of DPPH (150µm) was prepared in ethanol. 0.1 ml of various concentrations (31.25, 62.5, 125, 250, 500, 1000, 2000 µg/0.1 ml) of methanolic extracts and DPPH (1.9 ml) was added. Control is prepared in the same manner without the test compound. In blank, ethanol replaced DPPH. The mixtures were kept in dark for 20 mins, for the reaction to complete and absorbance was read at 517 nm. Lower the absorbance of the reaction mixture higher the scavenging activity. Quercetin (31.25, 62.5, 125, 250, 500, 1000, 2000 µg/0.1 ml) was used as standard. The percentage inhibition of DPPH was calculated as follows [7].

$$\% \text{ inhibition } = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

Nitric oxide scavenging activity

Nitric oxide scavenging activity of the extract was determined according to the method (green. et. al., 1982). Aqueous solution of sodium nitroprusside generates nitric oxide (NO) spontaneously at physiological pH. NO interacts with oxygen to produce nitrate ions which were measured colorimetrically. 3 ml of reaction mixture containing 2 ml of sodium nitroprusside (10 mM) in PBS and 1 ml of various concentration (62.5, 125, 250, 500, 1000, 2000 µg/ml) of methanolic extracts were incubated at 3Ȼ for 4 hours. Control without test compound was kept in the same manner. After incubation 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm [8, 19]. Quercetin (50, 100, 200µg/ml) was used as standard.

The percentage inhibition of nitric oxide scavenging was calculated using the formula,

$$\% \text{ inhibition } = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

Total anti-oxidant activity

Total anti-oxidant activity is a spectroscopic method used for quantitative determination of anti-oxidant capacity. Sample solution/ascorbic acid equivalent to 500µg/0.1 ml was mixed with 1 ml of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). In blank, 0.1 ml of methanol was used instead of sample. The reaction mixtures were incubated in a boiling water bath at 9Ɛ for 90 minutes. Samples were allowed to cool to room temperature. The absorbance of the reaction mixtures were measured at 695 nm against blank. The anti-oxidant activity was expressed as equivalents of ascorbic acid (µg/g) [20].

Determination of reducing power

For the measurement of reductive ability, transformation of Fe^{3+}-$Fe^{2+}$was investigated according to the method (Oyaizu. et. al., 1986) in the presence of 2.5 ml of phosphate buffer (pH 6.6), 2.5 ml of 1% potassium ferricyanide and extracts at different concentrations (31.25, 62.5, 125, 250, 500, 1000, 2000 µg/ml) were incubated at 50˚C for 20 minutes. To the mixture, 2.5 ml of 10% TCA were added and centrifuged at 3000 rpm for 10 mins. After centrifugation, 2.5 ml of the supernatant was diluted with 2.5 ml of water and added 0.5 ml freshly prepared 0.1% ferric chloride. The absorbance was measured at 700 nm [9]. The control solution consists of water in the place of sample. The increase in absorbance indicates higher reductive ability [10].

In-vitro anti-arthritic activity

0.5 ml of the reaction mixture consisted of 0.45 ml of 5%W/V bovine serum albumin in aqueous solution and 0.05 ml of various concentrations of crude extracts (31.25, 62.5, 125, 250, 500, 1000mcg/0.05 ml). 1 N HCl was used to adjust the pH to 6.3. The samples were incubated for 20 min at 37˚C and heated for 3 mins (57˚C). Cool the sample, add 2.5 ml of phosphate buffer. Turbidity produced was measured at 416 nm [11]. For test control, 0.05 ml distilled water was added. Bovine serum albumin was not present in the Product control [4]. The percentage inhibition of protein denaturation was given by

$$\% \text{ inhibition } = 100 - \frac{\text{OD of test solution} - \text{OD of product control}}{\text{OD of test control}} \times 100$$

Control indicates 100% protein denaturation. Diclofenac sodium was taken as standard.

Statistical analysis

Experiments were carried out in triplicate and results are given as the mean±S.D. deviation. All the data in all tests were analyzed using Microsoft Excel 2007.

RESULTS AND DISCUSSION

Anti-oxidant activity

DPPH scavenging activity

DPPH assay is rapid, easy and cost effective method to measure an antioxidant effect, which involves the use of the free radical [12], 2, 2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is commonly used to test the ability of compounds to act as free radical scavengers or hydrogen donors and it is based on the ability of DPPH to decolorize in the presence of antioxidant [5, 13]. Methanolic extract of *Caulerpa racemosa* showed a very good anti-radical activity in scavenging DPPH radical and showed a maximum % inhibition of 88.87±1.05% at 2000µg/0.1 ml concentration. DPPH scavenging activity of *Caulerpa racemosa* is shown in table 1.

Table 1: DPPH scavenging activity of *Caulerpa racemosa*

Concentration (µg/0.1 ml)	Percentage inhibition (%)	
	Caulerpa racemosa	Ascorbic acid
2000	88.87±1.05	98.04±0.62
1000	77.96±1.40	90.36±0.68
500	68.84±0.80	90.10±0.65
250	67.23±1.40	87.84±1.00
125	62.59±0.64	86.5±0.54
62.5	54.37±0.45	81.97±0.82
31.25	10.40±0.57	64.82±0.87

µg/ml**:** Microgram/milliliter

Nitric oxide scavenging activity

Nitric oxide scavenging activity is based on the inhibition of nitric oxide radical generated from sodium nitroprusside, which interacts with oxygen to produce nitrate ions and measured colorimetrically by the use of griess reagent [8, 14]. The methanolic extract of green algae confirmed the scavenging of nitric oxide radical, formed as a result of addition of sodium nitroprusside. NO scavenging activity was found to be increased with increase in concentration. It showed the maximum inhibition of 80.49±1.43% at 2000µg/ml. NO scavenging activity may be due to the anti-oxidative effects of flavonoids and tannins. Nitric oxide scavenging activity *Caulerpa racemosa* is shown in table 2.

Total anti-oxidant activity

Total anti-oxidant activity was assayed by Phosphomolydenum method through formation of Phosphomolydenum complex [15]. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH [16, 17]. It is a quantitative method since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid [18]. Total anti-oxidant activity of *Caulerpa racemosa* was found to be 32µg equivalents of ascorbic acid. From the results obtained, it is clear that the methanolic extract of *Caulerpa racemosa* possess free radical scavenging activity through total anti-oxidant activity. The total anti oxidant activity of *Caulerpa racemosa* is given in table 3.

Table 2: Nitric oxide scavenging activity *Caulerpa racemosa*

Sample	Concentration (µg/ml)	Percentage inhibition (%)
Caulerpa racemosa	2000	80.49±1.43
	1000	75.69±1.18
	500	71.06±0.78
	250	61.44±1.01
	125	60.94±0.81
	62.5	20.7±0.41
Quercetin	200	36.48±1.45
	100	21.99±0.82
	50	11.48±0.59

Table 3: Total anti oxidant activity of *Caulerpa racemosa*

Sample	Concentration (µg/ml)	Absorbance
Ascorbic acid	2000	0.0475±0.0001
	1000	0.0462±0.0011
	500	0.0426±0.0032
	250	0.0406±0.0005
	125	0.0275±0.0007
	62.5	0.0267±0.0006
	31.25	0.0233±0.0005
Caulerpa racemosa	500	0.0151±0.0008

Table 4: Effect of *Caulerpa racemosa* on reductive ability

Concentration (µg/0.05 ml)	Percentage inhibition (%)	
	Caulerpa racemosa	**Ascorbic acid**
2000	88.80±0.98	95.7±0.52
1000	79.70±0.99	95.59±0.52
500	74.582±1.0	91.09±0.84
250	65.44±0.65	89.3±0.55
125	56.41±0.89	79.77±1.15
62.5	43.37±0.34	73.96±0.55
31.25	6.64±0.26	70.81±0.55

Table 5: *In vitro* anti arthritic activity

Concentration (µg/0.05 ml)	Percentage inhibition (%)	
	Caulerpa racemosa	**Diclofenac sodium**
1000	49.33±0.597	98.37±0.075
500	44.91±0.805	98.27±0.055
250	43.36±0.405	98.19±0.061
125	18.87±0.341	98.14±0.072
62.5	18.01±0.208	94.47±0.193
31.25	14.33±0.575	93.98±0.331

Determination of reducing power

The reducing capacity of a compound may serve as a vital recorder of its potential anti-oxidant activity. For the estimation of reductive ability, transformation of Fe^{3+}-$Fe^{2+}$was investigated. The change in the optical density of the mixture is measured at 700 nm [9]. An increase in optical density indicates higher reductive ability [10]. Thus, the results obtained revealed reductive ability of *Caulerpa racemosa* and it was found to be increased with rise in concentration. Table 4 shows the reductive ability of *Caulerpa racemosa*

Anti-arthritic activity

The methanolic extract of *Caulerpa racemosa* produced a significant inhibition of protein denaturation and its effect was compared with standard Diclofenac sodium. It showed maximum percentage inhibition of 49.33±0.597% at 1000mcg/0.05 ml. Denaturation of proteins and production of autoantigens is the major cause of rheumatoid arthritis [4]. From the results obtained, it can be stated that the methanolic extract of *Caulerpa racemosa* is capable of controlling the production of autoantigens and thereby inhibit the denaturation of proteins. The *in vitro* anti arthritic activity of *Caulerpa racemosa* is given in table 5.

CONCLUSION

The *in vitro* activity of methanolic extract of *Caulerpa racemosa* revealed that it has potential dose dependent anti-oxidant and anti-arthritic properties. Steroids, triterpenoids, alkaloids, tannins, glycosides, carbohydrates, flavonoids and proteins are present in *Caulerpa racemosa* and these might be responsible for bringing out its potentiality in having these properties. The results obtained are encouraging to carry out the structural exploration of the anti-oxidant and anti-arthritic components present in them. Further studies are required to signify the mechanism of action of these pharmacological activities and to mark them out for their significant pharmacological actions.

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CONFLICTS OF INTERESTS

Declared None

REFERENCES

1. Heo SJ, EJ Park, KW Lee, YJ Jeon. Antioxidant activities of enzymatic extract from brown sea weeds. Bioresour Technol 2005;96:1613-23.
2. Heinz Lullmann, Albrecht Ziegler, Klaus Mohr. Color atlas of pharmacology. 2nd ed; 2000. p. 320.

3. Goodman, Gilman. Manual of Pharmacology and Therapeutics. McGraw-Hill companies; 2008. p. 54.
4. L Shravan Kumar N, Kishore G, Siva Kumar G, SindhuPriya ES. *In-vitro* anti-inflammatory and anti-arthritic activity of leaves of Physalis angulata. Int J Pharm Ind Res 2011;1(03):211-3.
5. https://seaweedindustry.com/seaweed/type/caulerpa-racemosa
6. Blois Ms. Antioxidant determination by the use of stable free radical. Nature 1958;181:1199-200.
7. Sanchez Moreno C, Larraurij A, Saura-calixto FA. Free radical scavenging capacity of selected of selected red rose and white wines. J Sci Food Agric 1990;79:1301-4.
8. R Lavanya, S Umamaheshwari, G Harish, J Bharath Raj, S Kamali, D Hemamalini, *et al*. Uma Maheshwara Reddy. *In-vitro* anti-oxidant studies of Anisomeles malabarica and Coldenia procumbene L. Res J Pharm Biol Chem Sci 2010;1(4):737-44.
9. Polterait O. Antioxidants and free radical scavengers of natural origin. Curr Org Chem 1997;1:415-40.
10. Repetto MG, Liesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcer. Braz J Med Biol Res 2002;35:523-34.
11. Satoskar RS, Bhandarkar SD, Anipure SS. Pharmacology and pharmacotherapeutics, In, Pharmacotherapy of gout. 18th edi. Rheumatoid arthritis and osteoarthritis; 2003. p. 1022-7.
12. Richaupadhyay, Jitendra kumar chaurasia, Kavindra Nath Tiwari, Karuna Singh. Antioxidant property of aerial parts and root of phyllanthus fraternus webster, an important medicinal plant. Scientific World J 2014;2014:2-5.
13. MR Saha, SMR Hasan, R Akter, MM Hossain, MS Alam, MA Alam, MEH Mazumder. *In vitro* free radical scavenging activity of methanol extract of the Leaves of mimuso pselengi linn. Bangl J Vet Med 2008;6(2):197–202.
14. Palash Mandal, Tarun Kumar Mishra, Mitali Ghosal. Free radical scavenging activity and phytochemical analysis in the leaf and stem of Drymaria diandra Blume. Int J Integr Biol 2009;7(2):80-4.
15. Mediacano A, Alcaraz O, Arnao MB. Free radical scavenging activity of indolic compounds. Anal Bioanal Chem 2003;376:33-7.
16. Oyaizu M. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Japan J Nutr 1986;44(6):307–15.
17. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E. Anal Biochem 1999;269:337-41.
18. Raghu KL, Ramesh CK, Srinivasa TR, Jamuna KS. Total antioxidant capacity in aqueous extracts of some common vegetables. Asian J Exp Biol Sci 2011;2(1):58-62.
19. S Banerjee, A Chanda, A Ghoshal, R Debnath, S Chakraborty, R Saha, *et al*. Nitric oxide scavenging activity study of ethanolic extracts of from two different areas of kolkata ixora coccinea. Asian J Exp Biol Sci 2011;2(4):595-9.
20. Abubakar B Aliyu, Mohammed A Ibrahim, Aliyu M Musa, Aisha O Musa, Joyce J Kiplimo, Adebayo O Oyewale. Free radical scavenging and total antioxidant capacity of root extracts of Anchomanes difformis Engl. (araceae). Acta Pol Pharm 2013;70(1):115-21.