Research Article

# Screening of Phytochemicals, Antibacterial, Antioxidant and Anti-inflammatory Activity of *Dictyota barteyresiana* Seaweed Extracts

**Swarna Bharathi Durairaj, Boopathy Raja Andiyappan**

Department of Zoology, Nehru Memorial College (Autonomous), Puthanampatti, Tiruchirappalli, Tamil Nadu, INDIA.

Submission Date: 22-02-2020; Revision Date: 25-03-2020; Accepted Date: 21-04-2020

## ABSTRACT

To evaluate the antibacterial, antioxidant and anti-inflammatory activity of various extracts of *Dictyota barteyresiana.* Five strains of gram positive and gram negative bacteria such as *Proteus vulgaris, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus* and *Klebsiella pneumoniae* could be inhibited by plant extracts through disc diffusion method. The results were supported that antibacterial activity of ethanolic extract shows inhibition zone comparatively higher than other extracts. Antioxidant activity demonstrated in two various ways DPPH and Phosphomolybdenum assay from this ethanolic extract reduces free radical scavenging during tissue oxidation. Protein denaturation assay were performed for investigating anti-inflammatory method. Ethanol extract exhibits highest action over tissues which slightly best results than other samples. The present study is focused to establish the novel drugs from medicinal plant of *Dictyota barteyresiana* to get multiple characteristic features.

**Key words:** *Dictyota barteyresiana,* Secondary metabolites, Antioxidant activity, Anti-inflammatory activity, Antibacterial activity.

**Correspondence:**
***D. Swarna Bharathi,***
Department of Zoology,
Nehru Memorial College,
Puthanampatti--621007,
Tiruchirapalli, Tamil Nadu,
INDIA.
Phone no: +918682016879

Email: swarnaraj93@gmail.com

## INTRODUCTION

The wide variety of seaweed present in submerged oceanic waters as inhabitant area. They are classified as Chlorophytes, Rhodophytes and Phaeophytes based on their chemical constitution and structure. *Dictyota barteyresiana* is a kind of rare seaweed that belongs to Phylum Phaeophytes and multicellular in nature. Mostly phaeophytes species are submerged under the seawater which forms unicellular and multicellular architecture. They possess respiratory and phytochemical pigments such as Chlorophyll a and c, Beta carotene, Lutein, Fucoxanthin and Dioanthin. Generally seaweeds have enormous potentiality against the abnormal growth, viral cells and bacterial cells even they inhibits the growth of intestinal worm.[1-3] There are plenty of experiment shows seaweed having an excellent biological effect such as antibacterials, anti-inflammatory and antioxidant activity.[4,5]

Seaweed extracts can play efficient role for inhibiting uncontrollable cell growth of human cancer cell lines.[6] The omnipresent nature of macroalgae might stimulates interesting over the researchers to examined antibacterial activity in different geographical regions.[7-12] Cyclic polysulfide's and halogenated compounds are essential vulture of disease causing pathogens. Brown macroalgae varieties exhibit significant antibacterial activity in the methanolic extract whereas red and green algae require acetone for providing best results. The six edible seaweed from Ireland show strong antioxidant as well as antimicrobial activity.[13] Seaweeds are good ancient medicine for treating goiterand prevention of scurvy which used by Japanese and Chinese.[14]

The various extracts of *Dictyota barteyresiana* shows the presence of essential phenolic compounds such as Alkaloids, Steroids , Phenols, Flavonoids, Saponins, Tanins, Glycosides, Sugars.[15] These are the bioactive materials which can enhance the antibacterial activity

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DOI : 10.5530/ajbls.2020.9.4

of natural compounds. In the health point of view, seaweed contains more amounts of minerals, proteins and few amount of lipids.[16-18] Antioxidant is a kind of capacity to eradicates the free radical which induces lipid peroxidation and can change the biomolecule structure further it may damage cellular organelles and causing cancer. Age related diseases and degenerative diseases are formed due to the formation of free radicals. There are number of disease caused by the causative agent of free radicals. This formation is easily restricted by natural compounds.

The Pharmaceutical drugs have side effects and stimulate inflammation to sensitive hosts. There are only a few ranges of natural product can releasing free radicals from the body. Oceans are constantly exposed to broad spectrum of free radicals in and out. It can eliminate the free radicals through certain characteristic feature beyond that inhabiting of seaweed inside the ocean could help the reaction to eliminating of ROS. Certain countries may cultures the seaweed as sea vegetables for enhancing industrialization and exported to foreign countries.[19]

## MATERIALS AND METHODS

### Sample Collection

*Dictyota barteyresiana* is brown algae and it prefers submerged portion of ocean column. For the experiment, it was collected from Mandabam at Kanyakumari district on January 2018 and April 2018 (Figure 1) and was washed in running tap water for 15 mins which is followed by removing unwanted soil residues through soaking. Distilled water is finally used for getting purified seaweeds. fter few days, samples were introduced into shade dry for 3 days which took long duration for drying when compared to other plants. The dried samples were powdered using a blender. (Authentication details : BSI/SRC/5/23/2019/Tech/3070)

### Extract Preparation

The plant materials were soaked into various solvents such as methanol, ethanol, benzene and acetone for preparing good quality of extracts. 25 grams seaweed powders was mixed with 250ml of each solvents and the mixture was left in a rotary shaker for 24hrs. The resulting mixture was filtered through Whitman filter paper. Some unwanted residues are removed through this filtration process and extracts were preserved for future use.

### Phytochemical Screening

The various extracts of *Dictyota barteyresiana* were analyzed for the presence of Resins, Amino acids, Gums, Glycosides, Tannins, Saponins, Steroids, Terpenoids, Alkaloids, Phenols, Flavonoids, Proteins and Carbohydrates according to the standard methods.[20,21] (Figure 2)

### Antioxidant Activity

Antioxidant activity of examined extract were determined by the two various method such as 2,2 diphenyl-1-Picryl-Hydroxyl assay and Phosphomolybdate assay.

#### *Dpph Method*

The 0.5ml, 0.10ml, 0.15ml of each extract were mixed with different concentration of DPPH solution followed by adding 2 ml of ethanol. The solution mixture kept at dark place for 30 minutes and ethanol used as blank (Figure 3). The absorbance of each sample was read at 517nm in spectrophotometer with some modification from standard methods.[22]

$$\% \text{ inhibition} = \frac{A - B}{A} * 100$$

A = Optical density of Sample

B = Optical density of Blank

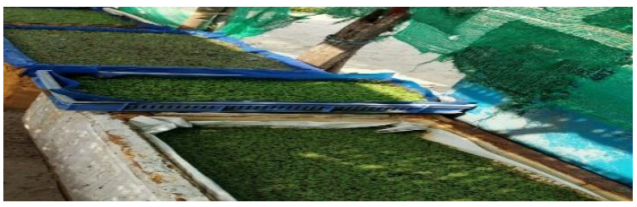

**Figure 1: Sample collection from Mandabam, South coast of India.**

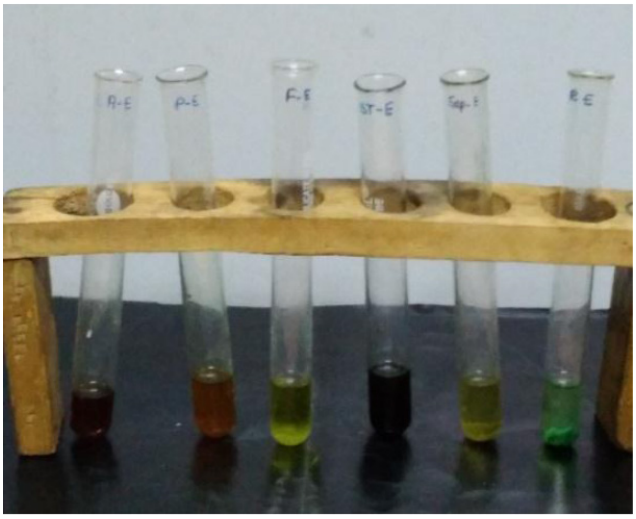

**Figure 2: Phytochemical screening of *Dictyota barteyresiana* ethanolic extract indicates colour changes.**

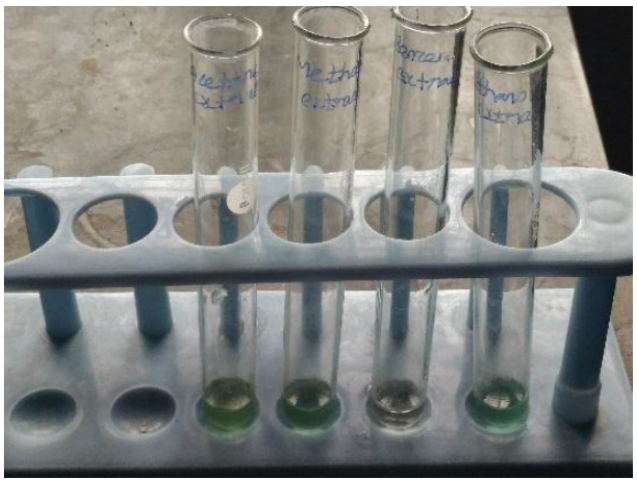

**Figure 3: DPPH method to determination of antioxidant activity in the various extracts of *Dictyota bartayresiana*.**

### Phosphomolybdenum Assay

The antioxidant activity was determined by the Phosphomolybdenum method. Reagent was synthesized by the mixture of 0.6M $H_2SO_4$, 28mM Sodium phosphate and 4mM ammonium molybdate dissolved into distilled water with known concentration. The various extracts of *Dictyota barteyresiana* were taken in each test tube which contains 3ml of distilled water and 1ml of Phosphomolybdate solution. Each sample mixture was kept in incubation at 95°C for 90 min resulting sample cooled at room temperature. After 30 min, absorbance is measured at 695nm in spectrophotometer.[23]

### Measurements of Antibacterial Activity

Both Gram positive and Gram negative bacteria were obtained from stock cultures of Microbiology laboratory. The bacteria which inoculated in culture plate named as *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Proteus vulgaris*. Nutrient agar commonly used as simple solid medium for their inducing ability of bacterial population.

### Disc Diffusion Method

Agar of known concentration was added along with distilled water in a flask and boiled. The boiling media was immediately poured into culture plate before solidification. Three various strains of *Proteus vulgaris, Pseudomonus aeruginosa, Bacillus subtilis, Staphylococcus aureus* and *Klebsiella pneumonia* were spread into culture vessels. 100µl of sample disc was inoculated in culture plate. The agar plate was incubated in incubator in 37°C. Labelling of concentration helps in obtaining the results. Zone of inhibition is measured after 24 hr.[24]

### Anti-inflammatory Activty

The 1ml, 2ml and 3ml of each extract was added with similar concentration of bovine serum albumin which was incubated at 37°C for 30 min. 5ml of phosphate which act as buffer was added to each sample and the OD value was read at 660nm in spectrophotometer. Control prepared the similar reagents without adding of bovine serum albumin.[25]

### Statistical analysis

All results are expressed as Mean values ±S.D of experiments conducted at least three times. Statistical significance was calculated using one way ANOVA.

## RESULTS

Amino acids and gums are absent in all extracts. In ethanolic extract the presence of Glycosides, Saponins, Flavonoids, Phenols, Alkaloids and Carbohydrate is found. The methanolic extracts have shown a significant presence of Tannins, Saponins, Flavonoids, Phenol, Carbohydrates and Proteins. Benzene extract contained only three phenolic compounds such as Resins, Steroids and Terpenoids whereas acetone extract indicated the presence of Glycosides, Tannins, Saponins, Steroids, Terpenoids and Alkaloids. Among the four samples ethanol extract shows highest phenolic content when compared to other extracts (Table 1).

Free radical scavenging capacities of living cells are induced by the presence of phenolic compounds in medicinal plant. There are plenty of phytochemical compounds found in nature among these flavonoids shows an excellent antibacterial, antioxidant and anti-inflammatory activity.[26] The ethanolic and methanolic

**Table 1: Phytochemical compound in *Dictyota barteyresiana* extract.**

| Compound | Ethanol Extract | Methanol Extract | Benzene Extract | Acetone Extract |
|---|---|---|---|---|
| Resins | – | – | + | – |
| Amino acids | – | – | – | – |
| Gums | – | – | – | – |
| Glycosides | + | – | – | + |
| Tannins | – | + | – | + |
| Saponins | + | + | – | + |
| Steroids andTerpenoids | – | – | + | + |
| Flavonoids | + | + | – | – |
| Phenols | + | + | – | – |
| Alkaloids | + | – | – | – |
| Carbohydrates | + | + | – | – |

extract exhibits more similarities of phenolic compounds which possess antibacterial, anti-inflammatory and antioxidant characteristic features. Methanolic and acetone extract have been found to tannin compound essential for inhibiting bacterial growth. Scavenging hydroxyl compounds and reducing inflammation of allergic consequences[27] (Table 2).

All the extracts except benzene show the presence of saponin compound which carries various biological activities important for therapeutic purposes.[28] Antioxidant activity is a primary source of valid drug in the medicinal world. Nowadays, beverages have been manufactured based on their free radical scavenging nature. The drugs must be possessing inevitable features for treating acute and chronic diseases, especially cardiac related diseases that are caused due to inappropriate action of scavenging systems which results of cellular damage. DPPH method shows percent of inhibition over free radical scavenging which represents ethanolic extract has been found 69.94 ± 0.01, 77.97 ± 0.01, 90.17 ± 0.01 inhibition whereas methanol extract exhibits 60.71 ± 0.01, 64.28 ± 0.02, 69.29 ± 0.07 lower than ethanoic results. Benzene and Acetone extract slightly varied from former observations such as 51.78 ± 0.01, 60.12 ± 0.01, 66.66 ± 0.01 and 56.25 ± 0.01,64.88 ± 0.01, 74.1 ± 0.01 respectively (Table 3).

Phosphomolybdenum assay to some extent proves than the previous methods that ethanol could inhibits 49.24 ± 0.02, 59.89 ± 0.02, 82.45 ± 0.02 which is higher than that of other samples acetone 32.33 ± 0.02, 45.48 ± 0.02, 49.24 ± 0.02 benzene 17.29 ± 0.02, 25.44 ± 0.01, 29.2 ± 0.01 and methanol49.24 ± 0.02, 64.28 ± 0.02, 69.29 ± 0.07 (Table 4). In pharmaceutical industry, some medicinal plants are used for scavenging free radicals and preventing tissue injury which are vital role in therapeutic purposes.[29] In present study supported that *Dictyota bartayresiana* of ethanol extract indicates highest free radical scavenging capacity. Protein denaturation assay were performed for understanding anti inflammatory activity of brown macroalgae. Ethanolic extract shows 56.45 ± 0.02, 69.35 ± 0.02, 71.50 ± 0.01 in different concentration of sample in other hand other extract depicts the 40.32 ± 0.02, 61.83 ± 0.01, 69.35 ± 0.02 of methanolic extracts. Finally benzene and acetone extract has lower level of anti-inflammatory capacity than others such as 37.09 ± 0.02, 50 ± 0.02, 56.45 ± 0.02 and 49.46 ± 0.02, 56.45 ± 0.02, 65.05 ± 0.01 respectively (Table 5).

*K.pneumoniae* species growth has been inhibited by ethanol (13mm), methanol (12mm), benzene and acetone (10mm). The *Dictyota barteyresiana* extract possess maximum inhibition zone against harmful pathogens, measurement carried out through mm which varies from solvent

**Table 2: Antibacterial activity of *Dictyota barteyresiana* against various pathogens.**

| Extracts | *Bacillus subtilis* | *Pseudomonas aeruginosa* | *Klebsiellapneumoniae* | *Staphylococcus aureus* | *Proteus vulgaris* |
|---|---|---|---|---|---|
| EE | 12mm | 16mm | 13mm | 17mm | 20mm |
| ME | 18mm | 14mm | 12mm | 15mm | 14mm |
| BE | 14mm | 11mm | 10mm | 8mm | 18mm |
| AE | 10mm | 8mm | 10mm | 6mm | 11mm |

**Table 3: Scavenging effect of *Dictyota barteyresiana* on DPPH method.**

| Concentration of extract in ml | Ethanolic extract | Methanolic extract | Benzene extract | Acetone extract |
|---|---|---|---|---|
| 0.5 | 69.94 ± 0.01 | 60.71 ± 0.01 | 51.78 ± 0.01 | 56.25 ± 0.01 |
| 0.10 | 77.97 ± 0.01 | 69.05 ± 0.01 | 60.12 ± 0.01 | 64.88 ± 0.01 |
| 0.15 | 90.17 ± 0.01 | 91.66 ± 0.01 | 66.66 ± 0.01 | 74.1 ± 0.01 |

**Table 4: Antioxidant assay of *Dictyota barteyresiana* extract investigating using the phosphomolybdenum method.**

| Concentration of extract | Ethanolic extract | Methanolic extract | Benzene extract | Acetone extract |
|---|---|---|---|---|
| 1ml | 49.24 ± 0.02 | 49.24 ± 0.02 | 17.29 ± 0.02 | 32.33 ± 0.02 |
| 2ml | 59.89 ± 0.02 | 64.28 ± 0.02 | 25.44 ± 0.01 | 45.48 ± 0.02 |
| 3ml | 82.45 ± 0.02 | 69.29 ± 0.07 | 29.2 ± 0.01 | 49.24 ± 0.02 |

**Table 5: Effect of various extracts of *Dictyota barteyresiana* over anti-inflammatory activity.**

| Concentration of extract | Ethanolic extract | Methanolic extract | Benzene extract | Acetone extract |
|---|---|---|---|---|
| 1ml | 56.45 ± 0.02 | 40.32 ± 0.02 | 37.09 ± 0.02 | 49.46 ± 0.02 |
| 2ml | 69.35 ± 0.02 | 61.83 ± 0.01 | 50 ± 0.02 | 56.45 ± 0.02 |
| 3ml | 71.50 ± 0.01 | 69.35 ± 0.02 | 56.45 ± 0.02 | 65.05 ± 0.01 |

to solvent ethanol (20mm), benzene (18mm), methanol (14mm), acetone (11mm). Ethanol shows highest inhibition against *Proteus vulgaris* when compared to others. Ethanolic extract (16mm) exhibits zone of inhibition followed by methanol(14mm), benzene (11mm) and acetone (8mm) against *P.aeruginosa*.

The *Bacillus subtilis* are rod shaped, gram positive bacteria which cause similar that of *E.coli* they inhibited in the form of ethanol (12mm), methanol (18mm), benzene (14mm) and acetone (10mm). The *Staphylococcus aureus* is also a kind of gram positive bacteria which damages respiratory tract. This is inhibited by ethanol (17mm), methanol (15mm), benzene (8mm) and acetone (6mm).

## DISCUSSION

Oxygen is a basic requirement for bacteria to metabolizing their enzymes. A bacterial membrane wrap with cell wall. It may be formed into various thicknesses. Gram positive strains surround with thick peptidoglycan layer whereas gram negative strains are inhabited within thin layer of peptidoglycan. This layer facilitates the uptake of oxygen into the cell.[30]

*Klebsiella pneumoniae* is a kind of Gram negative bacteria which causes various diseases in respiratory tract and urinary tract. The rod shaped *K. pneumoniae* found everywhere in the world. It does not require oxygen for their survival because *K. species* can thrive oxygen free environment, those species commonly known as facultative anaerobes. Mostly the people affected with diabetes are easily susceptible to *K. pneumonia* infection. Nowadays, *K. pneumoniae* resistance has greatly increased against certain antimicrobial agents.[31,32] Resistant strains of *K. pneumoniae* of human population could not be controlled by chemotherapeutic agents. It is a main reason for designing new drugs which alternative to antibiotic with good suppressor ability. *Proteus vulgar* is mainly found in human gut, fecal matter, soil and contamination of water. This species are chiefly responsible for urinary tract infection one or another way.[33] The indole group containing *p. vulgaris* have the capability to produce potential urease enzyme. It becomes the key enzyme for converting urea into ammonia and carbon dioxide respectively. The abnormal amount of ammonia may form struvite stone in the kidney when *P.vulgaris* invade human body would mainly target the immune system. Long term hospital inhabitant patients are prone to *P.vulgaris* infections.

*Pseudomonas aeruginosa* mainly resides in the lungs and kidney. These types of Gram negative bacteria cause urinary infection in a huge range. Patients with cystic fibrosis are mostly susceptible to *P.aeruginosa* infections. Apart from that, infections could not be completely exterminate if once enters into patient with cystic fibrosis.[34] The person without cystic fibrosis would be affected by *P.aeruginosa* infection as sudden onset, whereas cystic fibrosis patients fight against *P.aeruginosa* in a prolonged interval.[35] In recent days, this bacterial strain does not have sensitivity to antibiotics which reveal that mutations occurred in *P.aeruginosa* extreme fast manner, when compared to past decades. Intracellular enzymes of *P. aeruginosa* may change for each corresponding mutations.[36] The detrimental nature of certain pathogen growth must be suppressed by medicinal plants of their phenolic compounds which proved through inhibition measured at mm.

Our present study has revealed that ethanolic extract has peak level inhibition against both gram positive as well as gram negative bacteria which reduces free radicals and enhancement of antioxidant activity. The results of Anti-inflammatory activity have proved that acetone extract is more effective than other three samples such as ethanol, methanol and benzene. The anti-inflammatory activity of *Dictyota barteyresiana* is due to the presence of certain phenolic compounds like tannins, flavonoids and saponins which suppress free radical action during inflammation.

## CONCLUSION

In our present study it is proved that ethanolic extract of *Dictyota barteyresiana* possess antioxidant, anti-inflammatory and antibacterial activity. Further study is needed to establish the production of promising drugs for pharmaceutical purposes which are natural compounds that prevent the other side effects in consumers.

## ACKNOWLEDGEMENT

Thank you to Mr. Rajendra Kumar and the Algae Research Center for the assistance in securing the permit to collect seaweed and for being supportive in the conduct of this study. The authors would also like to thank SRM Institute of Science and Technology

## CONFLICT OF INTEREST

Authors declare no conflict of interest.

## ABBREVIATIONS

**DPPH:** 2,2-diphenyl-1-picrylhydrazyl.

## SUMMARY

This study reports on the phytochemical constituents in the various seaweed extracts of *Dictyota barteyresiana* collected from Mandabam, Tamil Nadu. The antibacterial, antioxidant and anti-inflammatory properties of the extracts were also determined. Results of the showed the presence of Resins, Glycosides, Tannins, Saponins, Steroids, Terpenoids, Alkaloids, Phenols, Flavonoids, Proteins and Carbohydrates in seaweed extracts of *Dictyota barteyresiana*. The biological activities of ethanolic extracts were consistently higher than that of the other solvents. Furthermore, the seaweed can be promising sources of natural antibacterial, antioxidant and anti-inflammatory compounds.

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**Cite this article:** Durairaj SB, Andiyappan BR. Screening of Phytochemicals, Antibacterial, Antioxidant and Anti-inflammatory Activity of *Dictyota barteyresiana* Seaweed Extracts. Asian J Biol Life Sci. 2020;9(1):20-6.