Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 2081-2089 | ISSN:2251-6727

An Invitro Analysis of Antioxidant Potential Of Acanthophora Species Using Ethanolic Crude Extract

Urvi Echhpal
Postgraduate student
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Chennai, India
Siva Perumal
Associate Professor
Department of Prosthodontics,
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Chennai, India
Dhanraj Ganapathy
Professor and Head
Department of Prosthodontics,
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Chennai, India
Vaishnavi Rajaraman
Assistant Professor
Department of Prosthodontics,
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Chennai, India
Corresponding author
Vaishnavi Rajaraman
Assistant Professor
Department of Prosthodontics,
Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences,
Saveetha University,
Chennai, India

(Received: 02 September 2023 Revised: 14 October Accepted: 07 November)

KEYWORDS
acanthophora, seaweed , antioxidant activity, oral pathogens, plants, health, seagrasses

ABSTRACT

Aim: This study was performed to investigate antioxidant activity of Acanthophora species**.**

Background: Marine algae is consumed in many regions of the world. Natural product usage for medicinal reasons is an ancient science, and minerals and products from different plants and animals were the main sources of medicines for a very long time. Free radicals damage proteins, lipids, and DNA and cause a variety of illnesses in people. Therefore, using antioxidants from an external source can help manage this oxidative damage.

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 2081-2089 | ISSN:2251-6727

Materials and Method: The extract was made using 200g of acanthophora , species of red algae, which was then placed in a water bath at 60 degrees celsius for 24 hours , after which it was boiled down. The extract was then tested at different concentrations by DPPH assay, H2O2 assay, NO assay to assess antioxidant potential.
Results: The study showed that acanthophora species showed appreciable antioxidant potential. All the assays performed showed significant values proving the same.
Conclusion: There have been found to be good antioxidant properties within the limitations of the present study, further quantitative analysis use be done to test the same.

INTRODUCTION

Acanthophora belongs to the family of red algae. Red algae is nothing but type of seaweed. The main use of seaweed in India is to make phycocolloids, which are useful for industrial and commercial sector [1] Seaweeds are a diversified category of macroalgae that may be roughly divided into different categories based on the presence of photosynthetic pigments as rhodophyta (red algae), chlorophyta (green algae), and phaeophyta (brown algae) [2]. Seaweed extracts contain both major and minor minerals, amino acids, vitamins, cytokinins, auxin, and compounds that behave like growth-promoting agents [3]. Popular seaweeds include agar, carrageenan, and alginate; these have been utilized as food for humans, plant fertilizers, and sources of numerous chemicals [4].

Seaweeds have been shown to have antibacterial and antinociceptive properties. Nature has always been a tremendous source of relief for humans, always providing cures from its plants, animals, and other sources.[5] The plant kingdom contains a variety of plants that have medicinally valuable compounds that have yet to be discovered. With an increasing number of individuals seeking treatments and health approaches devoid of negative effects produced by synthetic medicines, medicinal plants are shifting from the fringe to the mainstream [6]. Many plant secondary metabolites, particularly those from seaweed, are employed frequently as antioxidants. Infections were frequently treated by our ancestors using plants in the form of crude extracts or decoctions .[7] Therefore, it is crucial to identify the phytochemical components in order to understand the type of biological activity that the plant may display.[8]

Studying the antioxidant activity of this plant's other sections is thus an intriguing topic that may lead to the development of treatments for illnesses that affect both humans and animals. Another issue that directly impacts humans is bacteria that cause dental caries and result in tooth structure damage. Therefore, learning how to employ bioactive substances from plants to prevent harmful bacteria will be helpful information for replacing chemicals and antibiotics as well as avoiding chemical residues in dentistry [9].

Antioxidants from an external source can help manage this oxidative damage, that is caused by free radicals.[10] Therefore, the purpose of this work is to conduct a preliminary phytochemical screening of acanthophora bark, leaf, pod, and twig in order to ascertain their efficacy against pathogenic bacteria in the oral cavity.

MATERIALS AND METHODS

The study was conducted in the Green lab of Saveetha Dental College, with approval from Institutional Review Board of the university.

SAMPLING AND PRE PROCESSING

Leaves and barks of *acanthophora* were collected from Gulf of Mannar biosphere reserve, Tamil Nadu. The samples were washed thoroughly with tap water then shade dried on table tissue paper for 4 weeks . [8,11]The collected leaves and bark were washed under tap running water and dried in an incubator at 40°C. Using an electric blender, dried leaves and bark were ground into fine homogeneous powders, which were then steeped in three different solvents (95% ethanol, methanol, and chloroform) at room temperature in the dark for three days (Figure 2). Each sample was filtered through Whatman® No. 1 filter paper (Whatman International, England) and the filtered solutions were then evaporated to dryness using water evaporator at 40°C. The plant extracts were dissolved in dimethyl sulfoxide (DMSO). [12]

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 2081-2089 | ISSN:2251-6727

Figure 1: Acanthophora samples collected

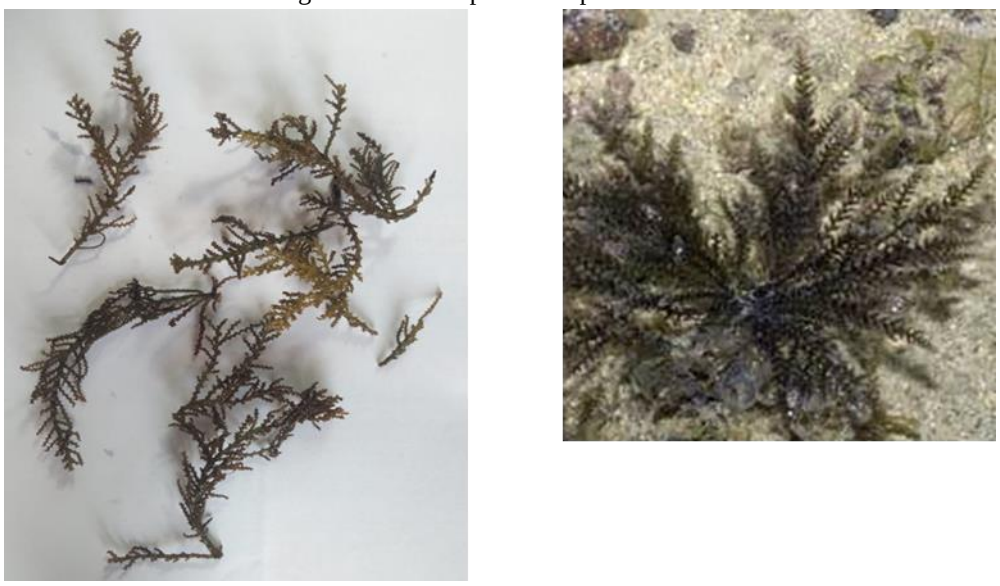


Figure 2 : The dried powdered form of Acanthophora samples after treatment

ANTI-OXIDANT TESTING

DPPH (1, 1-diphenyl-2-picrylhydrazyl) ASSAY

The antioxidant potentials of leaves and ethanol, methanol and chloroform extracts (10 mg/ml) were

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 2081-2089 | ISSN:2251-6727

studied using the paper disc diffusion method [13]. DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay was performed to evaluate the radical scavenging activity (RSA) of the synthesised extract. BHT (Butylated hydroxytoluene) was taken as the standard for the evaluation (control group). About 100 µl of respective samples of plant extract were added to all tubes marked as tests (test groups) except one which received BHT (control group). 200 µl of DPPH reagent was added to all the test tubes including blank and all the test tubes were incubated at room temperature in dark condition for 30 min. The absorbance were read at 517 nm wavelength and the anti-oxidant activity was calculated using the given formula:

% of antioxidant activity= [(Ac−As) ÷Ac] × 100

where: Ac—Control reaction absorbance; As—Testing specimen absorbance.

Hydrogen peroxide scavenging assay

The ability of the extract to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of Ruch et al. Aliquot of 0.1 mL of extracts (25–400 µg/mL) was transferred into the eppendorf tubes and their volume was made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4) followed by the addition of 0.6 mL of H_2O_2 solution (2 mM). The reaction mixture was vortexed and after 10 min of reaction time, its absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. The ability of the extracts to scavenge the H_2O_2 was calculated using the following equation:

$$\%\text{Inhibition} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100\%$$

NO Scavenging Activity

Sodium nitroprusside was used for generation of NO and it was measured by the Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride (NED), and 3% phosphoric acid). It. spontaneously generates NO in aqueous solution at physiological pH and results in generation of nitrite ions by its interaction with oxygen, whose estimation is done by Griess reagent. Scavengers of NO compete with oxygen leading to reduced production of NO. (Ganapathy et al. 2020)Different concentrations (100–1000 µg/mL) of plant fractions dissolved in ethanol and water was mixed with SNP (10 mM) in phosphate buffer saline (PBS) and incubated at 25°C for 3 h. The samples were then reacted with griess reagent, and absorbance was recorded at 546 nm of chromophore formed as result of diazotization of nitrite with sulphanilamide, and subsequent coupling with NED was done using microplate reader and compared to positive control which in this case was ascorbic acid treated in same way to Griess reagent. The ethanol was used as control using the following formula:

$$\text{Nitric oxide scavenged}\,(\%) = \frac{(A_{\text{control}} - A_{\text{test}})}{A_{\text{control}}} \times 100.$$

Statistical analysis

The results were tabulated in Microsoft Excel (Microsoft Corporation, Redmond, WA) and exported to SPSS version 22.0 software (IBM Corp., Armonk, NY) for statistical analysis. The present data were represented as Mean ± SD, while One Way Analysis of Variance (ANOVA), was carried out between the control and experimental acanthophora species extract at 10 µl, 20 µl, 30 µl, 40 µl, and 50 µl concentrations. Any p-value less than 0.05 was considered significant.

RESULTS

DPPH Assay

The following is the percentage of inhibition with standard deviation for various concentrations of the extract using DPPH assay (Table 1). The results of the present study showed that the acanthophora species using ethanolic crude extract had better anti-oxidant potential at lower concentrations while the anti-inflammatory potentials were slightly higher than the controls in higher concentrations. The highest anti-inflammatory activity was 80% at 50 µl ($p = 0.000$). In comparison with control, the highest anti-inflammatory activity was noted at 10 µl ($p = 0.002$) (Table *1* and Figure *3*).

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 2081-2089 | ISSN:2251-6727

Table 1 : Table showing the percentage of scavenging for various concentrations of the extract using DPPH assay

CONCENTRATION OF EXTRACT	% OF INHIBITION±S.E
20µg/ml	11.28±2.60
40µg/ml	29.53±2.90
60µg/ml	40.27±2.50
80µg/ml	59.15±2.40
100µg/ml	74.09±2.50
120µg/ml	81.64±2.90

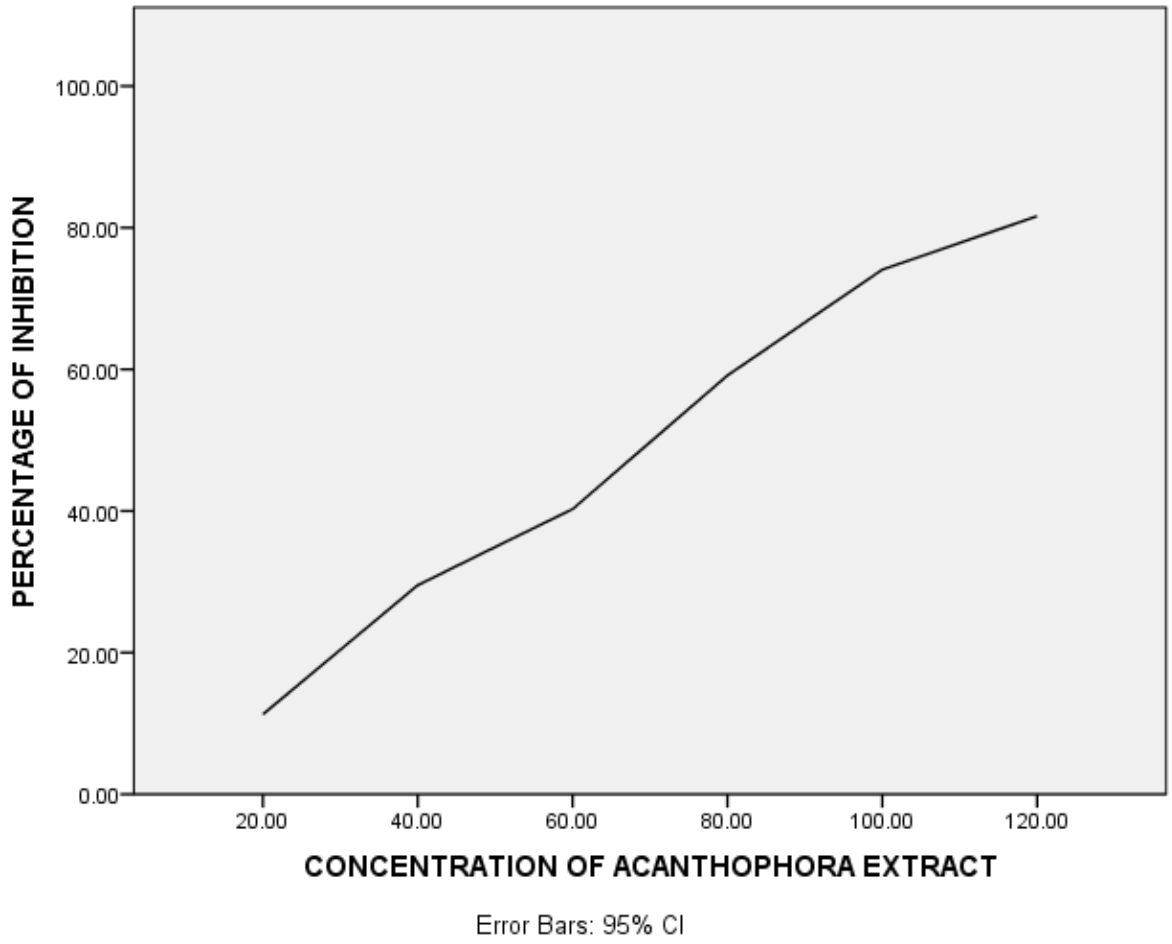


Figure 3:

The percentage of scavenging for various concentrations of the extract using Hydrogen peroxide assay (Table 2). The results of the present study showed that the acanthophora species using ethanolic crude extract had better anti-oxidant potential at lower concentrations while the anti-inflammatory potentials were slightly

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 2081-2089 | ISSN:2251-6727

higher than the controls in higher concentrations. The highest anti-inflammatory activity was 80% at 50 µl (p = 0.000). In comparison with control, the highest anti-inflammatory activity was noted at 10 µl (p = 0.002) (Table *2* and Figure *4*).

Table 2: Table showing the percentage of scavenging for various concentrations of the extract using h2o2 assay

CONCENTRATION OF EXTRACT	% OF INHIBITION±Standard Error
20µg/ml	19±2.70
40µg/ml	32±3.10
60µg/ml	50±2.80
80µg/ml	58±2.50
100µg/ml	69±3.00
120µg/ml	76±2.40

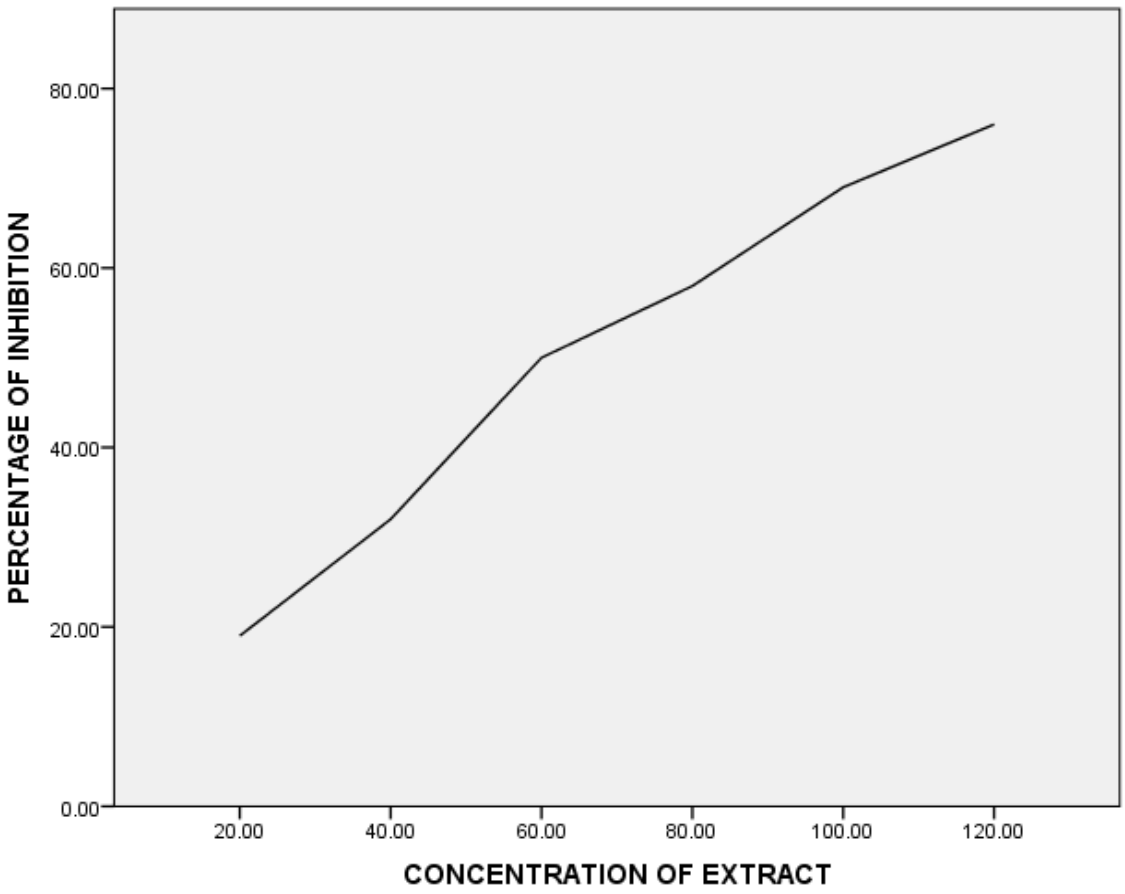


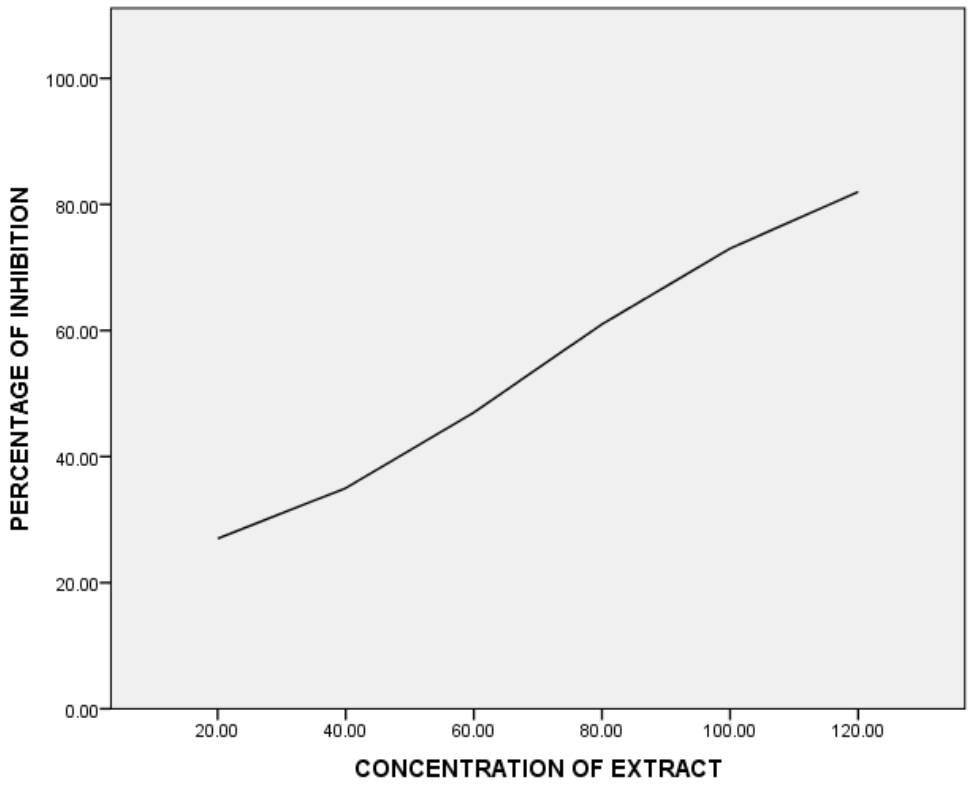
FIGURE 4:

Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 2081-2089 | ISSN:2251-6727

The percentage of scavenging for various concentrations of the extract using DPPH assay (Table 3). The results of the present study showed that the acanthophora species using ethanolic crude extract had better anti-oxidant potentialat lower concentrations while the anti-inflammatory potentials were slightly higher than the controls in higher concentrations. The highest anti-inflammatory activity was 80% at 50 µl (p = 0.000). In comparison with control, the highest anti-inflammatory activity was noted at 10 µl (p = 0.002) (Table *3* and Figure *5*).

Table 3: Table showing the percentage of scavenging for various concentrations of the extract using NO assay

CONCENTRATION OF EXTRACT	% OF INHIBITION±Standard Error
20µg/ml	21±2.70
40µg/ml	35±3.10
60µg/ml	47±2.80
80µg/ml	61±2.50
100µg/ml	73±3.00
120µg/ml	82±2.40



Journal of Chemical Health Risks
www.jchr.org
JCHR (2023) 13(4), 2081-2089 | ISSN:2251-6727

Discussion still not adequate and relevant to study. Kindly discuss the study results in proper context. Do not add generic and redundant sentences. Also, expand the discussion.

DISCUSSION

Research on antioxidants is essential to the scientific investigation of substances that can lessen the negative consequences of oxidative stress. In in vitro research, ascorbic acid demonstrates its versatility by playing a crucial role in preserving cellular health, examining oxidative stress and redox reactions, and enhancing our understanding of a variety of cellular processes. Cell biology, neurology, immunology, and drug discovery are among the study areas where it is significant . Reactive oxygen species (ROS) generation and the body's natural antioxidant defenses are out of balance, which causes oxidative stress . Extensive research into natural substances with antioxidant qualities has been prompted by the possible health effects of oxidative stress, including its link to aging, chronic diseases, and cellular damage.[14]

CONCLUSION

The results of the present in vitro antioxidant assay showed that the ethanolic extracts of various parts of acanthophora had an effective antioxidant activity. . This activity was supported by bioactive components such as anthraquinone,flavonoids, saponins, phenolics, alkaloids, especially terpenoids which are only found in leaf and twig. These are well known to possess antibacterial and other therapeutic properties [17].

The present study provides evidence of antioxidant properties that correspond to the phytochemical study which showed the active ingredients in acanthophora. This species could probably provide alternative bioactive agents to mitigate the problems of dental caries currently proliferating in dentistry.

AUTHOR CONTRIBUTION

This work was carried out in collaboration among all authors. Author Urvi Echhpal and Dr. Sivaperumal carried out the assays and experiments of the study. Author Dr. Sivaperumal designed the experimental setup for this study. Dr. Vaishnavi and Dr. Dhanraj supervised the whole research work and corrected the manuscript draft. All authors read and approved the final manuscript.

The current study's findings clearly demonstrated that extracts from acanthophora exhibited antioxidant efficacy.

Based on an previous studies, acanthophora was found to show good anti microbial properties, hence making it a strong material to use in dental treatment. Yang et al in 2020. Studies were also performed on a class of seaweed, once processed in a centrifuge, and tested for cytotoxicity.[15]

This antioxidant action could be attributed to active components found in plant extracts.This antioxidant activity could be attributed to active chemicals found in this plant such as anthroquinone, terpenoids, flavonoids, saponin, phenolics, and alkaloids. Some of these phytochemical substances have already been shown to possess such activities [16].

Based on the findings, it is feasible to conclude that ethanolic extracts of various sections of acanthophora have promise as a source of antioxidant agents . Furthermore, the quantitative assessment of its phytochemical elements is critical for future research.

Limitations of this study

DECLARATION OF CONFLICTING INTERESTS

All authors have declared no varying interests.

FUNDING SOURCE

We thank Saveetha Dental College and Hospitals for allowing us to conduct this study at the prestigious Blue lab. The study was funded by Echhpal Dentistry but no conflicts of interest were reported.

REFERENCES

1. Fleurence J, Levine I: Seaweed in Health and Disease Prevention. Academic Press; 2016.
2. Jha B, Reddy CRK, Thakur MC, Umamaheswara Rao M: Seaweeds of India: The Diversity and Distribution of Seaweeds of Gujarat Coast. Springer Science & Business Media; 2009.
3. Website. Accessed: https://doi.org/10.1007/BF00207588. 10.1007/BF00207588
4. Raj CTD, Muthukumar K, Dahms HU, James RA, Kandaswamy S: Structural characterization, antioxidant and anti-uropathogenic potential of biogenic silver nanoparticles using brown seaweed.

Front Microbiol. 2023, 14:1072043.
5. Dias DA, Urban S, Roessner U: A historical overview of natural products in drug discovery. Metabolites. 2012, 2:303–36.
6. Bedlovičová Z, Strapáč I, Baláž M, Salayová A: A Brief Overview on Antioxidant Activity Determination of Silver Nanoparticles. Molecules. 2020, 25.: 10.3390/molecules25143191
7. Lobo V, Patil A, Phatak A, Chandra N: Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010, 4:118–26.
8. Kim S-K, Shin K-H, Venkatesan J: Marine Antioxidants: Preparations, Syntheses, and Applications. Academic Press; 2022.
9. Rathi CR, Suresh SN, Geethalakshmi S, Ilangovan M, Rasheed R, Vazhacharickal PJ: Synthesis of silver nanoparticles from Mirabilis jalapa and evaluation of antioxidant activity. Prem Jose;
10. Karunasiri AN, Gunawardane M, Senanayake CM, Jayathilaka N, Seneviratne KN: Antioxidant and Nutritional Properties of Domestic and Commercial Coconut Milk Preparations. Int J Food Sci. 2020, 2020:3489605.
11. Kim S-K: Marine Biochemistry: Isolations and Techniques. CRC Press; 2022.
12. Priyadharshini, Pandiar D, Shanmugam R, Poothakulath Krishnan R: An In Vitro Evaluation of Anti-inflammatory and Antioxidant Activities of Cocos nucifera and Triticum aestivum Formulation. Cureus. Published Online First: 11 November 2023. 10.7759/cureus.48649
13. Suwan T, Wanachantararak P, Khongkhunthian S, Okonogi S: Antioxidant activity and potential of Caesalpinia sappan aqueous extract on synthesis of silver nanoparticles. Drug Discov Ther. 2018, 12:259–66.
14. Aleebrahim-Dehkordy E, Rafieian-Kopaei M, Amini-Khoei H, Abbasi S: In Vitro Evaluation of Antioxidant Activity and Antibacterial Effects and Measurement of Total Phenolic and Flavonoid Contents of L. Fruit Extract. J Diet Suppl. 2019, 16:408–16.
15. Bhattarai HD, Paudel B, Lee HS, Lee YK, Yim JH: Antioxidant activity of Sanionia uncinata, a polar moss species from King George Island, Antarctica. Phytother Res. 2008, 22:1635–9.
16. Santhi K, Sengottuvel R: Qualitative and Quantitative Phytochemical analysis of Moringa concanensis Nimmo. International Journal of Current Microbiology and Applied Sciences. 2016, 5:633–40. 10.20546/ijcmas.2016.501.064
17. Kappelle M: Ecology and Conservation of Neotropical Montane Oak Forests. Springer Science & Business Media; 2006.