EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Coden USA: EJBPBO
Volume: 13
Issue: 2
Year: 2026

EXPLORATION OF ANTIBACTERIAL AND ANTIBIOFILM POTENTIALITY OF WOOD ALGAE FOUND IN TRIPURA

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How to cite this Article: Sangita Debnath[1], Supriya Debnath[1], Reshmi Chowdhury[1], Akhankha Paul[1], Debjani Bhowmik[2], Manash C. Das[1]*. (2026). Exploration of Antibacterial and Antibiofilm Potentiality of Wood Algae Found In Tripura. European Journal of Biomedical and Pharmaceutical Sciences, 13(2), 109–113.

Article Received on 05/01/2026 **Article Revised on 25/01/2026** **Article Published on 01/02/2026**

ABSTRACT
Escherichia coli, a Gram-negative bacterium that has intestinal and extra intestinal infections in human beings and also has resistance to most of the antibiotics used. In view of the growing need to develop natural antimicrobial agents, the following study was designed to test the antibacterial, antibiofilm, and minimum inhibitory concentration properties of wood algae extract in a laboratory setting. The laboratory experiment or practical part of the study was conducted using *E. coli* and laboratory procedures that involve the preparation of nutrient broth and nutrient agar, sub-culturing, CFU assay, Gram staining, antibiotic sensitivity test, zone of inhibition, broth microdilution, or MIC assay, and biofilm assay. The study found that the wood algae extract resulted in a significant inhibition of the growth of the bacteria in broth and agar, with low CFU and OD in some concentrations, including about 6 µL. Although no significant zone of inhibition was observed when compared to standard antibiotics like ciprofloxacin, the extract demonstrated a mild antibacterial and antibiofilm effect against *E. coli*. Overall, the findings suggest that wood algae water extract possesses limited but detectable antibacterial activity and may serve as a potential natural source for further antimicrobial research. The study highlights the need for advanced investigations to isolate active compounds and enhance their efficacy for possible applications in antimicrobial therapy.

KEYWORDS: Wood algae water extract, antibacterial activity, *Escherichia coli*, minimum inhibitory concentration (MIC), antibiofilm activity, natural antimicrobial agents.

INTRODUCTION
Escherichia coli is considered one of the most common Gram-negative bacteria, usually living in the human intestines, but it can also cause a wide range of intestinal and extra-intestinal infections-some even life-threatening. Long-term exposure has led to resistance, with many strains developing against generally used antibiotics, thus rendering treatment difficult. Wood algaes are gaining interest scientifically due to their nutritional value and are a source of bioactive compounds that have been known for their antimicrobial and antioxidant properties and as medicines. Wood algaes are traditionally used in foods and folk medicine; therefore, they are also being explored for their potential sources of natural antibacterial agents.[3] The objectives are to investigate the efficacy of wood algae water extract against *E. coli* through antibacterial, antibiofilm, and inhibitive studies by means of standard microbiological techniques. This study attempts to get insight from bacterial growth, colony formation, biofilm development, and minimum inhibitory concentration towards the potential role of wood algae extracts as a natural antimicrobial agent and to contribute to this search for alternative strategies in fighting bacterial infections safely and sustainability.[4]

The extract of wood algaes has a number of key advantages in scientific research and applications. One of the key benefits of the current research is its role in the quest for a natural and safer approach over chemical antibiotics, in particular at a point in time when bacterial resistivity to antibiotics, such as *Escherichia coli*, is escalating rapidly in the medical field. On the other hand, the natural sources of wood algae extracts are regarded as non-poisonous as well as environmentally friendly in comparison with chemical antibiotics. This particular research work will be of key importance in appreciating the medicinal aspects of common wood algaes, which have often been overlooked in their role as a nutritional supplement.[5] In addition, the research regarding the antibacterial and antibiofilm activities exerted by wood algae extracts can help understand the mechanism by which natural compounds can interact with bacterial growth and biofilm formation, two important elements in forming infections. Based on the results achieved in this research, new studies can be encouraged to isolate and improve the active compounds in wood algaes that can be used in producing medication. Furthermore, the research also reinforces the concept regarding the potential for sustainable and effective antimicrobials to be used in the medical, food, and biological industries. The research not only adds to the knowledge available but also promotes the utilization of natural elements for the betterment of human health in a safer and better manner.[6]

MATERIALS AND METHODS

Preparation of wood algae water extract

Fresh and healthy specimens of wood algaes were selected and washed under clean water to remove any dirt or impurities. The wood algaes were dried in air and cut into small pieces to enhance the surface area required for the extraction process. The pieces of wood algaes were subsequently ground to form a coarse paste. The ground wood algae paste was finally mixed with a predetermined quantity of distilled water and heated or left to sit under room temperature for an appropriate number of hours to allow the active constituents to dissolve in the water. This solution was later cooled and filtered using sterile filter papers to form the filtrate, which was used as the water extract of the wood algae in the antibacterial experiment.[1]

Preparation of bacterial culture media (Nutrient Broth)

First weight 1.8 gram of nutrient broth powder and put it in conical flask with a cotton plug. Then measure 120ml of distilled water in a measuring cylinder and add in conical flask containing nutrient broth powder. Dissolve the nutrient broth powder in the water through mixing. Autoclave it at 121°C with 15 psi pressure for 15-20 min in autoclave.[1]

Preparation of bacterial culture media (Nutrient Agar)

Suspend 1.8 of nutrient broth powder in 120ml of distilled water with 4.8gm of agar powder. Stir this mixture while to fully dissolve all components. Autoclave the dissolved mixture at 121°C at 15 psi pressure for 15-20 minutes. Once the nutrient agar is put into each plate and leave plate on the sterile surface until the agar has solidifies. Use this solid agar plate for preparing lawn and subculture of bacteria.[1]

Subculture of bacteria in nutrient broth

First prepare nutrient broth and keep in a conical flask, and then autoclave the nutrient broth at 121°C & 15 psi pressure for 15-20 min. After competition of autoclave, take out the conical & keep in laminar for coding upto room temperature. Take out the mother culture from the incubator and keep in laminar, after that mix the mother culture flask properly with micropipette. Inoculate 100µl of cell (from the mother culture) into the conical flask & mix properly. Incubate the conical flask in an incubator at 37°C for 48 hrs. After 48hrs, take out the conical and measure the OD of cell at brown taking freshly prepared broth as blank. OD at 600 nm determines the growth of bacteria.[2]

Subculture of bacteria in nutrient agar and measurement of Colony Forming Units (CFU)

First of cell sterilize all the instruments by using 70% isopropyl alcohol and clean all over the surface where experiment preparation will be done by using alcohol, then measure 100ml distilled water by measuring cylinder. By using electric balance machine measure 1.5gm of nutrient broth after measuring dilute the nutrient broth in 100 ml of distilled water which was measured previously in a beaker. Again by using electric balance machine measure 4gm of agar-agar powder and mix it with nutrient properly, after completion of mixing, put the mixture into conical flask and cotton plug it. Then wrap the conical flask, micro tip box, petridish with paper and wrap the cotton with aluminum paper. After that autoclave the conical flask micro tip box, cotton and petridish. During the time of autoclave, switch on the laminar or air flow unit and on blower, U.V for 30 minutes. Once the time of autoclave is done take out all the materials that have been put in the autoclave and kept these in laminar air flow chamber to cool down. Now light Bunsen burner and turn on the blower in the chamber. Take out the conical flask of nutrient agar and pour it in petridish. Slightly close the laminar air flow chamber leave the petridish to dry and settle down, after petridish becomes dry take out previous cultured bacteria and drop it in the middle of nutrient agar in petridish. Spread the drop all over the surface area of petridish clockwise and anticlockwise by this process make bacterial lawn with the help of cotton and forceps. After the lawn is done keep the petridish into the incubator for 48hrs to check the results.

Gram staining of bacteria

At first take a clean and grease free slide after that prepare the smear of suspension on the clean slide with a loopfulof sample. After preparing the smear air dry the slide. When the slide dry pour crystal violet solution and keep it for about 1 minute and rinse in water. Then pour iodine for 1 minute and wash with water, after that wash with acetone for about 10-20 seconds and rinse with water. Then add safranin for about 1 minute and wash with water. After that air dry or blot dry and observe under microscope.

Antibiotic sensitivity Assay

Suspend 1.8 of nutrient broth powder in 120ml of distilled water with 4.8gm of agar agar powder. Stir this mixture while to fully dissolve all components. Autoclave the dissolved mixture at 121°C at 15 psi pressure for 15-20 minutes. Once the nutrient agar is put into each plate and leave plate on the sterile surface until the agar has solidifies. After that mark 1, 2, 3, 4 in each plates and add gentamicin according to the required concentrations and incubate it for 48hrs. After that take the OD.[2]

Determination of minimum inhibitory concentration of unknown compound by broth microdilution method

First we will take 10 test tubes, then add egg albumin 10µl to each test tube and change each number of micropipette for 10µl of test tubes. After that add distilled water to the same test tube 10µl (1 to 10 test tubes). Then we will mix it well which has been added to 1 to 10 test tubes. Then check the absorbance of each test tubes through the colorimeter machine, before that we have to bring zero with the water filled test tube in the colorimeter machine. One unknown sample is taken and using the same technique and I have determine the absorbance of unknown sample.[2]

RESULTS

Preparation of wood algae water extract

The prepared water extract of the wood algae was obtained and used for the experiment. The water extract was combined with nutrient broth while the culture of *Escherichia coli* and the growth pattern were observed.

Collection of wood algae sample

Samples of wood algaes were obtained from local market. These samples obtained were used for gram staining to analyze and detect the presence of bacteria. Further, these samples obtained were used for bacterial lawn to measure the number of bacterial colonies present in wood algae and analyze bacterial motility. For all further experiments, these samples obtained were cultivated in LB broth at 37°C for 24 hours.

Figure 1: Conical flasks containing nutrient broth inoculated with culture of *E. coli* sample.

MIC of collected wood algae water extract

The results obtained from the MIC experiment on wood algae water extract demonstrated inhibition of growth for *Escherichia coli* at certain concentrations, with pronounced inhibition demonstrated at a concentration of 6µL. There was decreased optical depth at this concentration compared to other concentrations, which depicted the reduced growth of bacterial organisms. Although there is less inhibition exhibited than for ciprofloxacin, there is evidence of mild antibacterial properties demonstrated by the wood algae extract.

Concentration	Optical Density (OD)
3microlitre(A)	1.15
3microlitre (B)	1.22
4microlitre (A)	1.09
4microlitre (B)	1.05
5microlitre (A)	1.15
5microlitre (B)	0.96
6microlitre (A)	**0.65**
6microlitre (B)	**0.69**
7microlitre (A)	1.09
7microlitre (B)	0.92
Ciprofloxacin (A)	0.18
Ciprofloxacin (B)	0.16
Control	1.09
Blank (A)	0.05
Blank (B)	0.02
Standard (A)	0.1
Standard (B)	0.1

Gram staining of *E. coli* after treatment with wood algae water extract

Gram staining of *E. coli* was performed. It was observed that through gram staining bacterial samples appears gram negative with rod shape bacilli.

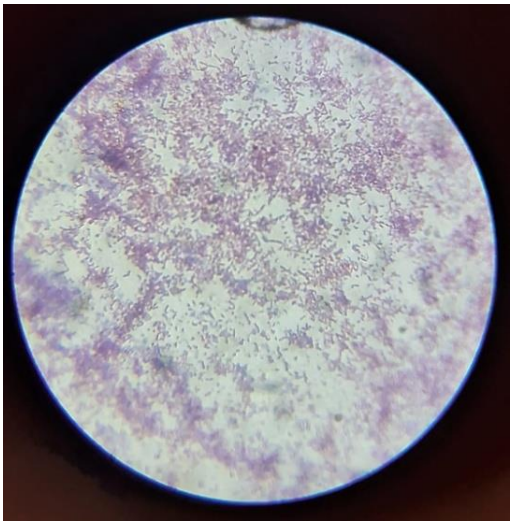

Figure 2: In this figure growth of bacteria after treatment with wood algae extract. Here the collected wood algae water extract treatment was given and bacteria were grown in broth culture for 48 hours at 37°C.

CFU count of *E. coli* after treatment with wood algae water extract

The result for the CFU count showed that there was a decreased growth in *Escherichia coli* when the wood algae water extract was applied. After 48 hours of incubation, only 92 bacterial colonies were obtained, showing fewer bacterial growth compared to the untreated culture. This indicated that the wood algae extract was capable of reducing the growth of the bacterium. Although the result was subtle, however, it supports the antibacterial property of the wood algae water extract.

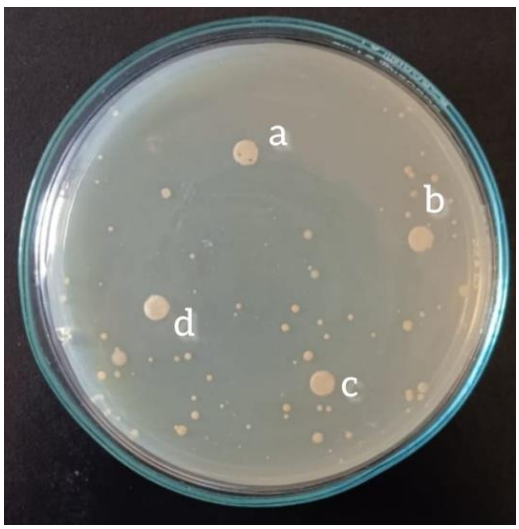

Figure 3: The petridish containing the concentration (a= Ciprofloxacin b= 5µL, c=6 µL, d=7 µL) of the wood algae extract displayed colonies, signifying mild antibacterial activity. The ciprofloxacin concentration displayed less colony growth than the wood algae extract, signifying relatively higher levels of antibacterial activity.

Antibiotic sensitivity assay after treatment with wood algae water extract

This was further corroborated with the result obtained from the antibiotic sensitivity assay that clearly indicated an un-distinct zone of inhibition created by the wood algae water extract against *Escherichia coli*, describing a very mild antibacterial effect. In contrast, ciprofloxacin exhibited a marked zone of inhibition devoid of bacterial growth, which agreed with its strong antimicrobial activity. Compared to ciprofloxacin, the wood algae extract showed less effectiveness but still had some inhibiting action toward bacterial growth.

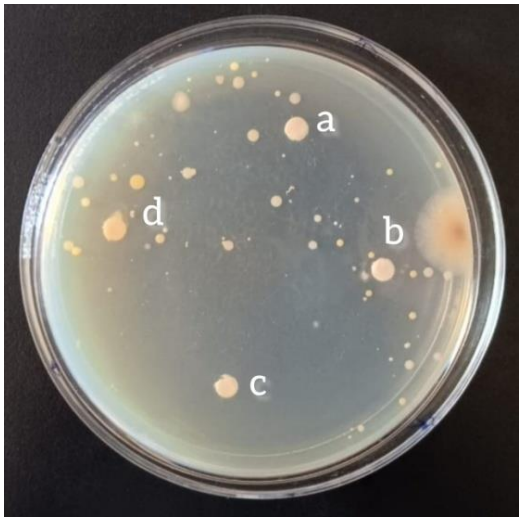

Figure 3: The agar plate containing the concentration of ciprofloxacin revealed a visible zone of inhibition, signifying a higher degree of antibacterial activity compared to the wood algae extract of concentration (a= 9µL, b=10µL, d= 8µL), which revealed least to no zone of inhibition.

DISCUSSION

The total antibacterial potential of wood algae water extract on *Escherichia coli* derived from observation results. The data from colony-forming unit count, MIC value, biofilm test, and antibiotic susceptibility test indicated that there was a mild inhibitory potential of wood algae extract on bacterial growth. The observable reduction in colony unit count and optical density readings indicated that this extract was able to reduce bacterial growth by slowing down bacterial replication, particularly at 6µL concentration that demonstrated maximum inhibition potential out of all tested doses. The lack of zone of inhibition on antibiotic susceptibility test result indicated that this inhibition potential of wood algae water extract was lower compared to ciprofloxacin antibiotic, which strongly inhibited bacterial growth.[9,10]

This phenomenon might have occurred due to lower concentration of antimicrobial agents present in this water extract or lower diffusion capability of present antimicrobial agents present in this water extract to diffuse in agar medium. This is further supported by the antibiofilm study, which observed that the wood algae extract could interfere with biofilm formation to some

extent, though not as effectively as conventional antibiotics.[7] Therefore, this generally points out that wood algae water extract, even though not performing as a potent antibacterial agent, does have measurable antibiotic and antibiofilm activities. These results are in line with the potential of wood algaes as natural sources for antimicrobial compounds and may suggest that higher concentrations, other ways of extraction, or active compound purification improve their efficiency against pathogenic bacteria such as *E. coli*.[8]

CONCLUSION

From above observation it can be concluded that the collected wood algae extract has antibacterial effect against *E. coli* at 6µL concentration. As the inhibition of *E. coli* growth after treatment with the extract was not significant, thus we can conclude that the extract execute minimum antibacterial effect against *E. coli*.

ACKNOWLEDGMENT

Authors would like to thank Dept. of MLT, Women's Polytechnic for instrumental support. All authors also like to acknowledge AICTE, Govt. of India for MODROB Project (F.No.9-114/IDC/MOD-POL/Policy-1/2021-22 dated 22.08.2022) to support by providing instrumental and chemical facilities of Microbial Biotechnology & Immunotechnology Lab, Dept. of MLT, Women's Polytechnic, Agartala, Tripura.

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