Uttar Pradesh Journal of Zoology

Volume 45, Issue 21, Page 148-152, 2024; Article no.UPJOZ.4293
ISSN: 0256-971X (P)

Evaluation of Antibacterial Activity of Solvent Extracts of *Spirulina fusiformis* against Pathogenic Bacterial Strains

G. Theivandran [a++*], J. Sakthibama [b++], A. Shajahan [b++] and L. Roselin Rajathi [b++]

[a] Department of Zoology, PTMTM College, Kamuthi-623604, Tamil Nadu, India.
[b] P.G. Department of Zoology, Pope's College, Sawyerpuram, Thoothukudi- 628251, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i214624

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/4293

Original Research Article

Received: 07/09/2024
Accepted: 10/11/2024
Published: 19/11/2024

ABSTRACT

This study explores the antibacterial properties of *Spirulina fusiformis*, a blue-green alga with significant dietary and medicinal value. *Spirulina* is recognized globally for its nutrient-rich composition and potential therapeutic applications. With antibiotic resistance emerging as a serious global health concern, algae-derived bioactive compounds offer a promising, natural alternative to traditional antibiotics due to their non-toxic profile and effectiveness against pathogenic bacteria. The antibacterial activity of *S. fusiformis* was assessed using various solvent extracts, including petroleum ether, benzene, ethyl acetate, methanol, and ethanol, tested against twelve bacterial

[++] Assistant Professor;
**Corresponding author: Email: deva.voc.net@gmail.com;*

Cite as: *Theivandran, G., J. Sakthibama, A. Shajahan, and L. Roselin Rajathi. 2024. "Evaluation of Antibacterial Activity of Solvent Extracts of Spirulina Fusiformis Against Pathogenic Bacterial Strains". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (21):148-52. https://doi.org/10.56557/upjoz/2024/v45i214624.*

strains, including *Bacillus thuringiensis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Escherichia coli*, using the well diffusion method. Results showed that the ethanol extract of *S. fusiformis* exhibited the highest antibacterial activity, inhibiting all tested bacteria, with the greatest zones of inhibition observed against *Streptococcus pyogenes* (17 mm) and *Bacillus thuringiensis* (16 mm). The presence of some significant antimicrobial compounds present in the ethanol extract. These findings support the use of *Spirulina fusiformis* as a natural antibacterial agent and underline its potential in developing alternative therapeutic strategies for combating antibiotic-resistant pathogens.

Keywords: Spirulina fusiformis; antibacterial activity and sea weeds.

1. INRODUCTION

Spirulina holds significant economic and health value due to its high nutrient composition and therapeutic potential. As a blue-green alga, *Spirulina* has gained popularity worldwide as a health food and functional supplement. It is a multicellular, filamentous cyanobacterium of the class Cyanophyta, noted for its rich nutrient profile that supports various health benefits (Bujard et al., 1970; Ganguli et al., 2009).

Antibiotic resistance in bacteria has emerged as a critical global health challenge, complicating the treatment of infectious diseases and leading to higher healthcare costs, prolonged hospitalizations, and increased mortality (Shajahan et al.,2022). This issue is exacerbated by the overuse and misuse of antibiotics in medicine, agriculture, and animal husbandry, fostering the growth of resistant strains and diminishing the efficacy of available treatments. Given this alarming trend, there is an urgent need to explore alternative therapeutic agents, including natural antimicrobials, to combat resistant pathogens and safeguard public health.

Algae are valuable natural resources with significant potential against pathogenic organisms. Globally, research has focused on identifying bioactive compounds derived from algae, as these natural compounds are typically safe, non-toxic, and effective without adverse side effects. In agriculture and aquaculture, algae-derived resources have demonstrated extensive beneficial activity. Crude algal extracts are essential for biomedical applications, with several studies evaluating their antimicrobial activity (Tuney et al., 2006; Moreau et al., 1998; Tang et al., 2002; Serkedjieva, 2004; Hellio et al., 2002). Notably, over 24,000 bioactive compounds with structural and functional diversity have been identified across various algal groups (Faulkner, 2001).

This study aims to compare the antibacterial efficacy of different solvent extracts of *Spirulina fusiformis* specifically petroleum ether, benzene, ethyl acetate, methanol, and ethanol against a range of pathogenic bacterial strains. By evaluating the antibacterial potential of these extracts, this research seeks to identify effective natural alternatives to traditional antibiotics, addressing the urgent need for novel antimicrobial agents in the face of rising antibiotic resistance.

2. MATERIALS AND METHODS

2.1 Algae Sources and Identification

Fresh and disease free algae of *Spirulina fusiformis* were collected from Antennae biotec lab commercial form, Kadachanendal, in Madurai. The algae was identified with the help of, Dr. Brindha, Associate Dean & Coordinator, CARISM Department, Sastra University, Thanjavur.

2.2 Bacterial Strains

The antibacterial activity of extracts of Petroleum ether, Benzene, Ethyl acetate, Methanol, Ethanol and Antibiotics against twelve species of bacteria namely*Bacillus thuringiensis, Bacillus subtilis, Streptococcus faecalis, Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis, Mycobacterium smegmatis, Salmonella paratyphi-A, Salmonella paratyphi-B, Salmonella paratyphi, Proteus mirabilis* and *Escherichia coli* were determined by well diffusion method. (Plate 1, Plate 2).

2.3 Well Diffusion Methods

The antibacterial activity was studied by well diffusion method. Circular wells of 6 mm diameter were prepared using well cutter. The wells have 25 µl holding

capacity. The wells were loaded with the crude and fractionated seaweed extracts and those loaded with the solvent alone served as control. Streak plate method was performed to seed pathogenic bacteria culture on the agar plates. Using the loop which had been flamed, cooled and dipped in the inoculums, continuous horizontal streaks were made in the solid agar plates. The clear labeling of sample was marked on the plates. The plates were then inverted and incubated at 37oC for 24 hours.

3. RESULTS AND DISCUSSION

The petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *S. fusiformis* were examined for the antibacterial activity against twelve pathogenic bacteria. Antibacterial activity on 12 different pathogenic bacteria showed that, ten bacterial strains were sensitive to petroleum ether and benzene extracts, nine bacterial strains for ethyl acetate and methanol extracts, and twelve for ethanol extract of S. fusiformis. The maximum zone of inhibition

Table 1. Antibacterial activity of *Spirulina fusiformis*

S.No.	Microorganisms	Name of the extract / Zone of Inhibition (mm)					
		Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol	AB
1	*Bacillus thuringiensis*	09	04	00	11	16	25
2	*Bacillus subtilis*	11	14	06	04	15	22
3	*Streptococcus faecalis*	12	14	06	04	13	21
4	*Staphylococcus aureus*	10	10	00	08	16	21
5	*Streptococcus pyogenes*	00	08	00	09	17	24
6	*Enterococcus faecalis*	11	12	06	04	09	22
7	*Mycobacterium smegmatis*	00	08	13	04	12	25
8	*Salmonella paratyphi-A*	08	00	08	00	07	18
9	*Salmonella paratyphi-B*	05	05	06	08	10	24
10	*Salmonella paratyphi*	09	10	07	08	13	26
11	*Proteus mirabilis*	08	00	12	00	09	24
12	*Escherichia coli*	07	12	08	00	06	19

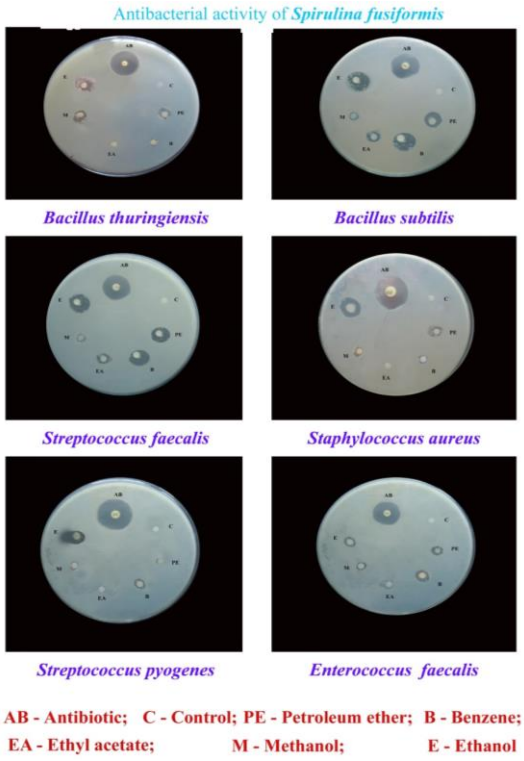

Plate 1. Antibacterial activity of *Spirulina fusiformis* against Gram-positive bacteria

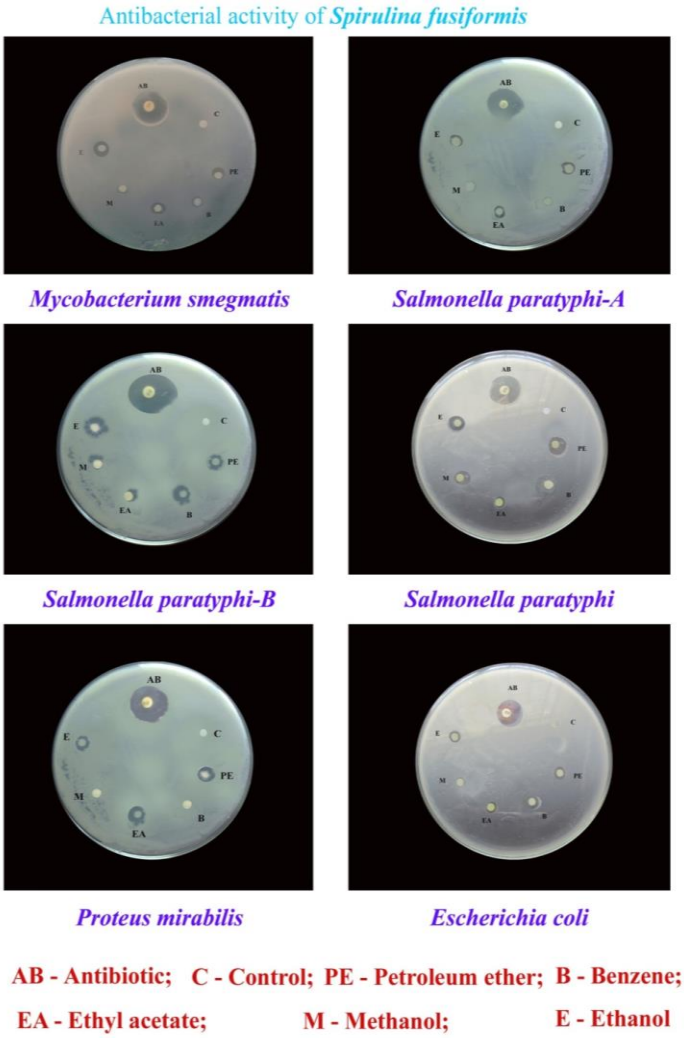

Plate 2. Antibacterial activity of *Spirulina fusiformis* against Gram-negative bacteria

17mm for *Streptococcus pyogenes* and 16mm for *Bacillus thuringiensis* and *Staphylococcus aureus* were observed against ethanol extract of *S. fusiformis* (Plate 1, Plate 2 & Table 1,). The ethanol extract of *S. fusiformis* showed highest activity (12/12) against the bacterial pathogens followed by petroleum ether, Benzene (10/12); methanol extract and ethyl acetate extract (9/12).

The antibacterial activity carried out against twelve pathogenic bacteria using column purified fractions of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *S. fusiformis*. The highest zone inhibition 17mm for *Streptococcus pyogenes* and 16mm for *Bacillus thuringiensis* and *Staphylococcus aureus* were observed against ethanol extract of *S. fusiformis* (Plate 1, Plate 2).

The ethanol extract of *S. fusiformis* showed highest activity against the all bacterial pathogens, because ethanolic extract of *S. fusiformis* have Heptadacane, octadecane, Eicosane, Hexadecane, phytol, Hexadeconic acid, and octadeconic acid major anti microbial compounds were identified by GC-MS analysis Diraman. H, et al., (2009), Ravi. M et al., (2010). In accordance with (Denfert andHube, 2007). their result to confirmed anti microbial activity of spirulina platensis.

4. CONCLUSION

The present study highlights the potent antibacterial activity of *Spirulina fusiformis* extracts against multiple pathogenic bacteria. Among the tested extracts, the ethanol extract demonstrated the highest efficacy, inhibiting all twelve bacterial strains. These findings align with

previous research on the antimicrobial properties of *Spirulina platensis* and reinforce the therapeutic potential of *Spirulina fusiformis* as a natural source of bioactive compounds. The study suggests that *Spirulina fusiformis* holds promise for further exploration in developing alternative antibacterial agents, especially in the context of increasing antibiotic resistance.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/4293