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RESEARCH ARTICLE

In vitro antibacterial activity of *Striga angustifolia* (D. Don) C. J. Saldanha against fish pathogens

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ABSTRACT

This study investigates the antibacterial properties of the *Striga angustifolia* medicinal plant extract against fish infections when combined with both antibiotic and non-antibiotic drugs. The produced extract was obtained from the entire *S. angustifolia* plant using various solvents, including ethanol, petroleum ether, and water. Extracts derived from *S. angustifolia* have been evaluated for their antibacterial properties against fish infections using the disk diffusion and well diffusion methods. The interaction between plant extracts and non-antibiotic medications was evaluated using the disk diffusion method. The ethanol extract exhibited significant efficacy against *A. caviae* (18mm) and *A. hydrophila* (17mm) in comparison to the petroleum ether extracts. *E. coli* exhibited a reduced level of inhibition from ethanol, measuring 12 mm. The petroleum ether extracts showed a maximum zone of inhibition of 17 mm against *E. aerogenes*. The petroleum ether extract of *S. angustifolia* exhibited a zone of inhibition of 8mm against *A. caviae* and *Vibrio cholera*. No substantial inhibition was detected from the aqueous extract of *S. angustifolia* against any of the test pathogens. Therefore, the results of this study show that these extracts might be useful for treating bacterial infections and stress how important it is to use plant extracts along with antibiotics and other drugs to control bacterial growth.

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Introduction

Plants act as efficient producers of active molecules and contribute to the production of several antibacterial substances. Medicinally significant herbs have a crucial function as an

appetizer, stimulant, in lipid metabolism, and for enhancing bodily power (Vaou et al., 2021). Additionally, it is purported to possess several therapeutic qualities, including antibiotic, anti-inflammatory, anti-diabetic, anti-hypertensive, anti-fertility, and anti-implantation effects. Furthermore, the entire pulverized plant was employed as a remedy for snake bites (Ojha et al., 2020). The process of identifying and separating the biologically active constituents from this medicinal plant could lead to the identification of new and potent molecules.

Striga angustifolia is a plant species classified under the family Fabaceae. It is alternatively referred to as senna or Indian senna. The plant has been utilized in traditional medicine for a range of functions, including as a laxative, anti-inflammatory, antidiabetic, and anticancer agent (Ghosh et al., 2011). *S. angustifolia* is a type of biomolecule that has anti-cancer properties. It primarily targets a specific category of plant hormones (phyto-hormones) (Hasan et al., 2018). Nevertheless, the comprehensive investigation of *S. angustifolia* for the identification of promising chemicals has not been fully conducted. There are still many unexplored areas relating to the potential impacts of plant chemicals (Jelin et al., 2015). This species has a vast natural distribution, encompassing regions in Africa, Asia, and the Middle East. This species is indigenous to the southern regions of Tanzania, the southern Arabian Peninsula, the Indian Subcontinent, Indo-China, and Hainan Island. Additionally, it has been introduced to various places, including Australia, America, and Europe.

Water serves as a habitat for various organisms, such as fish, saprophytic bacteria residing in sediments and plants, phytoplankton, and zooplankton. Certain organisms establish colonies on the skin, gills, and digestive tract of fish, coexisting with them in a mutually beneficial relationship. They aid in digestion and positively impact the immune systems of these animals. These germs can potentially harm fish health and are therefore referred to as conditionally harmful. The interplay between fish, bacteria, and diseases is a widely researched topic that has garnered global attention. The significant amount of attention these areas have received highlights their critical significance in fish disease (Picot et al., 2001). In spite of this, the spread of disease depends on many things, including how easily bacteria can cause illness, how fish's immune systems react, the conditions in the environment, and how strong the disease-causing agent is. Hence, alterations taking place in freshwater environments seem to have a crucial role in the emergence of various diseases, including newly identified ones (Bereded et al., 2021). Therefore, the current work aims to create several solvent extractions of *S. angustifolia* against different bacterial infections found in fish. Hence, it was deemed beneficial to examine this native flora for its efficacy against several bacteria.

Materials and Methods

Collection of S. angustifolia

Plant specimens were gathered from G. Venkataswamy Naidu College in Kovilpatti, Tamil Nadu, India, in June (Fig. 1A & B). The identification of plant specimens was facilitated by the Flora of Peninsular India, and the voucher specimens were stored in the herbarium center at the Indian Institute of Science in India. The plant materials were first washed with distilled water to eliminate soil impurities and then air-dried in a shaded area for one week.

Preparation of the plant extracts

The shade-dried powder (Fig. 1C) weighing 10g was placed in a Soxhlet device and extracted sequentially with 100 ml of ethanol (Fig. 1D), petroleum ether, and water for duration of 48 hours each. This extraction process was based on the modified Touzout et al., (2023) procedure. The extracts were subjected to vacuum evaporation at reduced pressure and thereafter stored in sterile glass vials at room temperature in the laboratory.

Bacterial culture and growth conditions

The microorganisms used in the tests came from the Department of Microbiology at Sri

Paramakalyani College in Alwarkurichi. They were *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter aerogenes, Aeromonas hydrophila, Aeromonas caviae,* and *Asiatic cholera*. Bacterial cultures are preserved exclusively on nutrient agar medium. The bacterial cultures are regularly subcultured and stored at 4°C to maintain their viability.

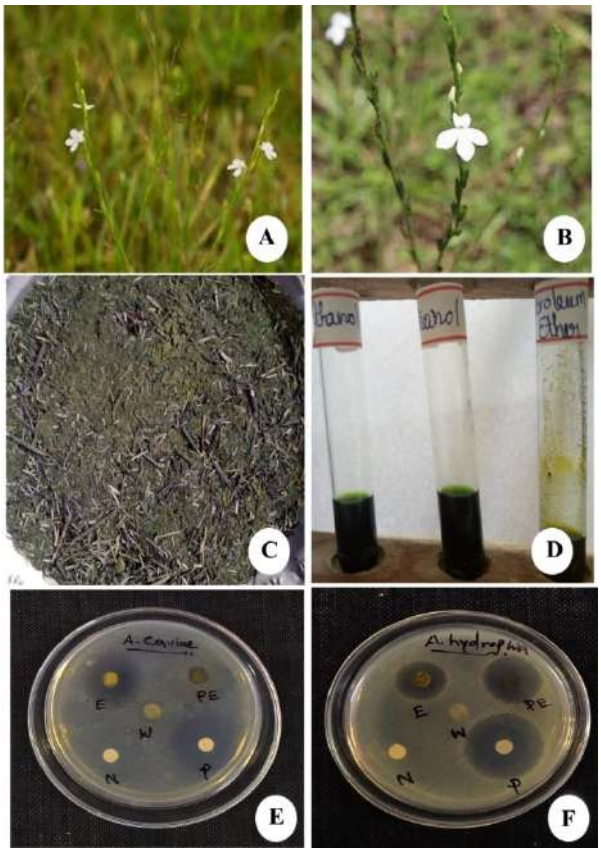

Fig 1. *S. angustifolia* plant (A), flower (B), plant dry powder (C), ethanol extract of *S. angustifolia* (D), anti-bacterial activity of plant extract against *A. caviea* (E) and *A. hydrophila* (F).

Plant extracts dilution an preparation of impregnated disc

The plant extracts were diluted in DMSO using a serial two-fold dilution method, starting with an initial concentration of 100 mg/ml, and distributed across a 96-well plate. Subsequently, 20 cc from each well was utilized to saturate a sterilized disc (Oxoid, UK) that served as a control. The test used a final concentration of 1 mg per disc. The impregnated discs were dried in a 37°C incubator for 24 hours and then promptly used for the experiment.

Disc Diffusion Method

The test bacteria were cultured at a temperature of 37°C for a duration of 8 hours using nutrient broth tubes. The broth cultures were applied over the surface of Muller-Hinton agar plates using sterilized cotton brushes. The plates were first dried and then utilized for the sensitivity assay. The impregnated plant extract disc is placed on the surface of the Mueller-Hinton agar plate. Each test plate contains six discs, including a positive control (Vancomycin 30 µg), a negative control (DMSO), and four discs treated with plant extracts. The control experiment consists of inoculums that do not contain any extract. The plates were incubated at a temperature range of 36–38 °C for a duration of 18–24 hours. The diameter of the zone of inhibition (in millimeters) was measured after the incubation period. The experiments were replicated three times, and triplicates were preserved. The mean values were documented (Hadacek & Greger, 2000; Nässel & Zandawala, 2020).

Results and Discussion

The ability of *S. angustifolia* extracts, such as ethanol, petroleum ether, and water, to kill seven types of bacteria that are known to be harmful to fish was tested in a lab setting. The antibacterial activity of ethanol, petroleum ether, and water extracts of the plant was tested against the bacteria, and the results are presented in Table 1.

The results show that the ethanol extract from *S. angustifolia* effectively stopped the growth of seven types of bacteria, with inhibition zones that were 12 to 18 mm in size. The largest zone of inhibition was seen in *A. caviea* measuring 18±0.51 mm (Fig. 1E). A 15±0.44 mm zone of inhibition was seen in the ethanol extract of *S. angustifolia* against *K. pneumonia* (Fig. 2 C), *A. hydrophila* (Fig. 1 F), and *V. cholerae* (Fig. 2 E). Fig. 2 A and B show that the ethanol extract of *S. angustifolia* had the smallest zones of inhibition against *E. aerogenes* and *E. coli*. These zones were 14±0.14 mm and 12±0.34 mm,

respectively. In addition, no substantial inhibition was seen for any of the extracts of *S. angustifolia* against *P. aeruginosa* (Fig. 2D).

Table 1. Antibacterial activity of *S. angustifolia* extracts against fish pathogen.

S. No.	Pathogens	Ethanol (mm)	Petrolium ether (mm)	Water (mm)	N (-) (mm)	P (+) (mm)
1.	*E. coli*	12±0.34	-	-	-	22±0.54
2.	*K. pneumonia*	15±0.44	-	-	-	16±0.64
3.	*P. aeruginosa*	-	-	-	-	20±0.35
4.	*E. aerogenes*	14±0.14	-	-	-	25±0.36
5.	*A. hydrophila*	15±0.54	17±0.24	-	-	25±0.39
6.	*A. caviea*	18±0.51	8±0.54	-	-	26±0.57
7.	*V. cholerae*	15±0.38	8±0.33	-	-	24±0.33

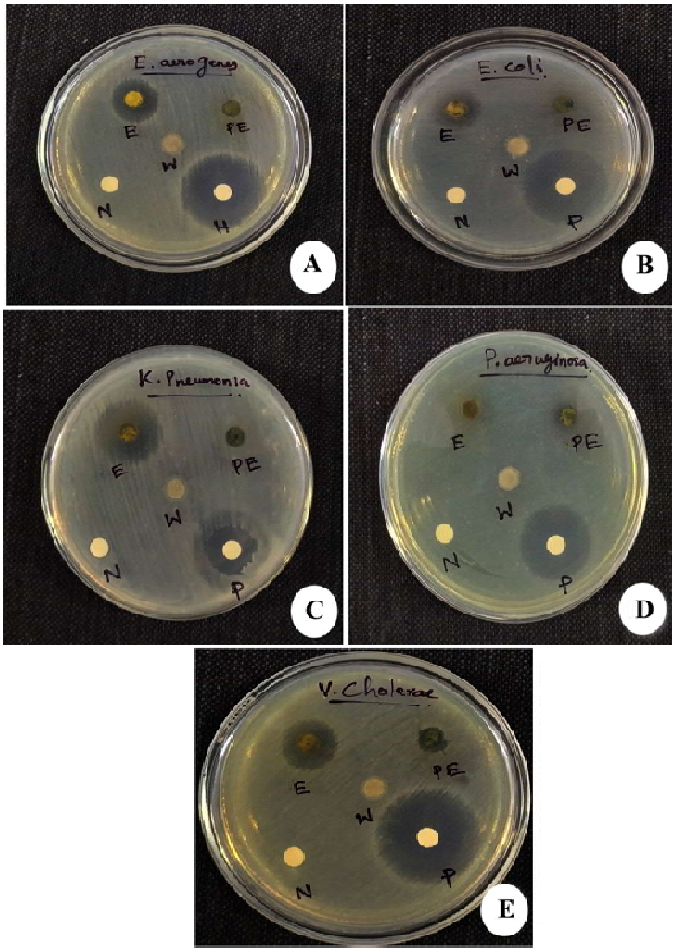

Fig. 2. Anti-bacterial activity of different extracts of *S. angustifolia* against *E. aerogenes* (A), *E. coli* (B), *K. pneumonia* (C), *P. aeruginosa* (D) and *V. cholera (E)*.

The petroleum ether extract of *S. angustifolia* exhibited an inhibition zone of 17±0.24 mm against *A. hydrophila*, 8±0.54 mm against *A. caviea*, and 88±0.33 mm against *V. cholerae*. No other test organisms exhibit considerable inhibition. Furthermore, the aqueous extract of *S. angustifolia* does not significantly inhibit all the test organisms. In addition, the ethanol extract of *E. angustifolia* did not exhibit any antibacterial activity against a specific Gram-negative bacterium (Hiremath et al., 2000). All test organisms have exhibited a response to the commercial antimicrobial agent according to the established standard.

Medicinal plants provide many limitations, such as limited availability and compliance with biodiversity conservation regulations. These challenges can be overcome by extracting weeds to isolate promising biomolecules. Various weed species, including as *Cuscuta campestris*, *Lantana camera, Chromolarna odorata,* and *Petiveria alliaceae*, have recently been studied for their antibacterial properties (Adesanya et al., 2023; Sangita Barik et al., 2020). When *A. salmonicida subsp. salmonicida* and most *A. sobria* strains were exposed to a dose of 0.01 mg/mL, their growth was slowed down. Similarly, for most *A. hydrophila* strains, the minimum inhibitory concentration (MIC) varied from 0.01 mg/mL to 0.19 mg/mL (Abdelhamed et al., 2019). Türker et al., (2009), revealed that the water-based extract obtained from the leaves of *F. vesca* possesses antibacterial properties against *A. hydrophila* and *Y. ruckeri*. In our investigation, only the ethanol and petrolium ether extracts of *S. angustifolia* were able to

suppress the growth of *A. hydrophila*. The essential oils of *M. longifolia* were found to suppress the growth of Listeria monocytogenes and *Klebsiella pneumoniae*, as described by (Messaoudi et al., 2021). As shown by Yassin et al., (2020), the methanolic extract of *M. longifolia* is effective against *S. aureus, Micrococcus luteus, E. coli,* and *Pseudomonas aeruginosa*.

The antibacterial activity of plant extracts from *S. angustifolia* shows that *A. caviae* and *A. hydrophila* were the strains that were least likely to be killed by ethanol and petroleum ether extracts, respectively. Compounds that exhibit potent inhibition of both indirect and directly acting mutagens typically demonstrate a pronounced capacity for scavenging free radicals (Xu et al., 2021; Zhou et al., 2021). Based on the aforementioned findings, it can be inferred that plant extracts possess significant promise in terms of their antibacterial efficacy against fish infections. Scientific support for the traditional use of this plant comes from *S. angustifolia*'s significant antibacterial efficacy. Our research indicates that the leaf of *S. angustifolia* has the potential to be a highly effective source of antibacterial properties. However, it is necessary to do additional research to identify the specific bioactive components that are responsible for the biological activity of *S. angustifolia.*

Conclusion

S. angustifolia is a botanical species that has been employed in traditional medicine for diverse therapeutic applications. *A. caviea, A. hydrophila, V. cholerae, K. pneumonia, E. aerogenes,* and *E. coli* are a few of the bacteria that can make fish ill that an ethanol extract of *S. angustifolia* has killed. Additionally, the Petrolium ether extract from *S. angustifolia* has demonstrated strong antibacterial activity against *A. hydrophila, A. caviea,* and *V. cholerae*, which are all bacteria that can make fish sick. Furthermore, the aqueous extract of *S. angustifolia* has shown no efficacy against any bacterial strain. The effectiveness of *S. angustifolia* in killing bacteria may vary depending on the type of solvent, concentration, and specific portion of the plant employed during the extraction process. The antibacterial action of S. angustifolia may be attributed to phytochemicals such as anthraquinones, flavonoids, tannins, saponins, steroids, and terpenoids. The inference drawn is that *S. angustifolia* exhibits promising antibacterial characteristics; however, further investigations are required to substantiate its effectiveness, safety, and mode of operation.

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