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Synergistic Effect Of Tridax Procumbens Leaf Extracts And Antibiotics Against Vibrio Parahaemolyticus Isolated From Fresh Water Shrimp

Dr. B. Nageshwari,[1] Dr. Masqood Ahmed Mohammad[2], Dr. D. Sailaja [3], K. Subhashini Devi[4], Dr. T. Sujatha [5]

1Lecturer in Biotechnology, Government College (Autonomous) Rajahmundry, B.nageshwari@gcrjy.ac.in.
2Lecturer in Microbiology, Government Degree College, Naidupeta, Tirupathi District, mdmaqsood.micro@gmail.com.
3Lecturer, Department of Zoology, Government College (Autonomous), Rajahmundry, sailaja123@gcrjy.ac.in
4Lecturer, Department of Zoology, Government College (Autonomous), Rajahmundry. subharenibagi@gcrjy.ac.in.
5Associate Professor, Department Of. Microbiology, Government College (Autonomous), Rajahmundry. tsujatha@gcrjy.ac.in

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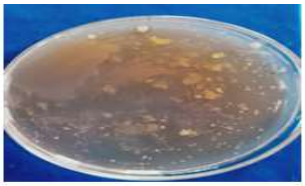
ABSTRACT

The main cause of human infections associated with seafood is Vibrio spp. One of the most commonly reported species is *Vibrio parahaemolyticus*. The antibacterial activity of methanolic extracts of Tridax procumbens against Vibrio parahaemolyticus, which was isolated from freshwater tiger prawns, was examined in this work. Additionally, we assessed the antibacterial activity of methanolic extracts of Tridax procumbens at varying doses against Vibrio parahaemolyticus, as well as their total phenolic and flavonoid contents. The growth of Vibrio parahaemolyticus isolated from prawns is efficiently inhibited by the synergistic impact of methanolic extracts of Tridax procumbens leaves combined with conventional antibiotics, with an inhibition zone ranging from 15 to 29 mm.

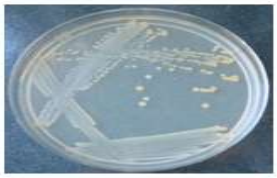
Key words: Shrimp, Antibacterial effect, Tridax procumbens, Synergistic effect.

Plant methanol extract

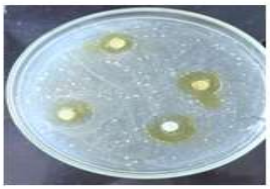
shrimp



Isolation plate



Vibrio spp. On NAM



Haemolysis

Antimicrobial activity

1. Introduction

The fastest-growing industry in the world's food production is aquaculture; between 1984 and 1995, the produced prawns and prawns grew at an annual pace of 16.8%. Shrimp production stands as a highly significant agricultural endeavor in Asia and South America, with widespread practices observed across the globe. Marine prawns represent a crucial product in the realm of international trade stemming from aquaculture. According to Di Maro et al. (2024), pond-reared Penaeid species account for about 26% of the overall product. Nonetheless, shrimp farming has faced a variety of considerable obstacles, including diseases affecting shrimp and inadequate practices, such as poor management of water quality. The most harmful diseases impacting prawns, leading to considerable economic losses for farmers, are caused by viruses and bacteria. [Abdlkareem, et al., 2023]. This kind of illness is brought on by bacterial infections, specifically those brought on by luminous Vibrio.

Shrimp stands out as a highly regarded seafood option, featuring prominently in the traditional cuisines of numerous nations. The widespread appeal has led to a global demand for its products. Given the constraints on wild catch supply, the cultivation of prawns has been initiated in numerous countries to satisfy demand, and this sector is experiencing significant growth. The growth can be assessed by examining the production figures from previous years, which increased from 1 million tonnes in 2001 to 4.02 million tonnes in 2010, with India's contribution being approximately 0.1 million tonnes. The growth potential of Indian prawns is demonstrated by a 24% increase in quantity and a 42% rise in value of its exports in 2012 compared to 2011. Shrimp production is projected to increase by over 50% worldwide between 2010 and 2030, highlighting its dynamic production capabilities. Fresh and pristine prawns can be presented either cooked or raw, accompanied by a sauce. From a nutritional perspective, shrimps are rich in protein, low in saturated fat and calories, and possess a neutral flavour. Given these attributes, shrimps serve as a natural enhancement in salads, pastas, curry, soups, and stir-fried dishes. Shrimps are recognised for their substantial content of vitamin B12, selenium, ω-3 highly unsaturated fatty acids (HUFA), and astaxanthin, which is a powerful natural antioxidant.

Shrimps exhibit lower fat content, reduced cholesterol levels, and a higher presence of polyunsaturated fatty acids compared to eggs. The prevailing insights regarding dietary cholesterol associated with egg consumption distinctly highlight the nutritional advantages of shrimps. Considering the evidence presented regarding health-related outcomes, the nutrient profiles examined in this study, and a thorough evaluation of the recommended dietary allowances set by national and international organisations, it can be concluded that prawns may be regarded as a healthy food option for humans. The health benefits of consuming shrimp extend beyond just ω-3 fatty acids and specific nutrients; they arise from the synergistic effects of the various nutrients found in shrimp, such as proteins, trace minerals, and carotenoids. Consequently, to gain from essential nutrients such as ω-3 HUFA, astaxanthin, protein, essential amino acids, vitamins, and minerals, individuals should incorporate a moderate amount of shrimp into their diet. (Syam et al., 2013).

Vibrio's significance in disease and mortality is acknowledged. Two species, V. cholerae and V. parahaemolyticus, are recognized as significant pathogens that impact human health. Additionally, it causes a number of fish diseases that pose serious problems for a large number of wild and farmed species. Pathogenic bacteria have been a crucial factor in this crisis. Vibrio species pose a significant threat in the context of bacterial infections affecting farmed prawns, with mortality rates soaring to 100%. Species such as V. harveyi, V. anguillarum, V. parahaemolyticus, and V. vulnificus are often linked to fatalities in hatcheries and grow-out ponds. This study focusses on the isolation and identification of Vibrio species from the infected hepatopancreas of P. vannamei (Fleischmann et al., 2022).

For the treatment of serious illnesses, phytopharmaceuticals remain an essential resource, especially in developing countries. About 60-80% of the worldwide population still relies on traditional medicine for addressing common health issues (Gupta, et al., 2020). There is hope for the creation of new antibiotics to treat drug-resistant illnesses since plants produce a large number of secondary metabolites, many of which have shown therapeutic promise and are a promising source of antibacterial agents. It is believed that these chemicals play a role in the plant's defence against infection by working in synergy with intrinsic antimicrobials (Hamad et al., 2021). The combination of plant extracts with antibiotics reduces the minimum inhibitory concentrations (MICs), improves synergistic activity, and consequently lessens side effects, economic costs, and sensory impact. The effectiveness of antibiotics against resistant bacterial infections (modifying agents) may also be improved by these combinations, which may successfully control some bacteria that show persistent resistance to antibiotics (Liu et al., 2020).

This study aims to evaluate the combined impact of the widely recognised medicinal plant Tridax procumbens on the pathogenic bacteria V. Parahaemolyticus. The goal is to enhance the efficacy of existing antibiotics, particularly the older and more affordable options, in combating resistant bacterial pathogens through the integration of plant extracts with these antibiotics.

2. Materials and Methods

2.1. Collection of samples

In the present study the **shrimp** samples were collected at local fish at Rajahmundry. The collected samples were transferred to laboratory and stored at refrigerator for further analysis.

2.2. Isolation of shrimp pathogenic Bacterial colonies

The samples undergo serial dilution at factors ranging from 10-1 to 10-9. The sterile TCBS- (Thiosulfate-Citrate-Bile salt-Sucrose) medium was prepared by sucking 0.1 ml of samples from 10-5, 10-6, and 10-7 and spreading them over the following: Peptone (10.00g), Yeast extract (5.00g), Sodium citrate (10.0g), Sodium thiasulphate (10.0g), Sodium cholate (3.0g), Oxgall (5.00g), Sucrose (20.0g), Sodium chloride (1.00g), Ferric citrate (1.00g), Bromo thymolblue-0.04g, Thymol blue-0.04g, Agar-14.00g, The final pH (at 20°C) is 8.6 ± 0.1, and 1000 cc of distilled water together with the petri plates are incubated overnight at 37-45°C. The incubation time was sustained for 24 to 48 hours at 37°C for the pathogenic culture.

2.3. Biochemical identification of shrimp bacteria:

The isolated bacterial species were identified by the following the morphological and biochemical characteristics of the individual colony was recorded. The individual colony was transferred to nutrient agar. The isolates were subjected to following different biochemical test such as Gram staining, Motility, Morphology, Indole test, Methyl red test, Pigmentation test, Voges proskauer test, Citrate test, Urease test, Lactose test, H2S production and starch hydrolysis as described by [Ahmed et al., 2020).

2.4. Antibiotic susceptibility test

Susceptibility of the bacterial isolates to seventeen antibiotics (Conc. µg/disc) (Amikacin, Amoxycillin, Amoxycillin/clavulanic acid, Azithromycin, Aztreonam, Cefotaxime, Chloramphenicol, Ciprofloxacin, Clindamycin, Nitrofurantoin, Norfloxacin, Oxacillin, Rifampicin, Streptomycin, Sulphamethoxazole/trimethoprim, Tetracycline and Vancomycin) was carried out by Kirby-Bauer disk diffusion technique according to Bauer et al. (1966). The antibiotic disks were purchased from HimediaCompany.

2.5. Plant sample collection and leaf extract preparation

The plant of Tridax procumbens leaves were collected in botanical garden of Department of Botany, Govt

College (A) Rajahmundry. After three days of shade drying, the plant leaves were successfully blended into a fine powder, then 10 g were extracted using an Methanolic solution (70% methanol and 30% deionized water v/v) in up to 100 ml, shaken at random at 100 rpm. Following a two-day incubation period, the extracted material was centrifuged for 30 min at 2.47×g before being filtered using standard filter sheets. In the end, extract lyophilization was performed at − 50 ◦C using a Telstar Model 50 in Barcelona, Spain, and the extracted materials were diluted in distilled water with specific recognized concentrations in mg/ml and stored for further analysis (Hamad, Hafez, & et al, 2023a).

2.6. Phytochemical analysis of plant materials

Standard protocols were used for the qualitative screening of phytochemical ingredients of methanol and ethanol extract. The presence of chemicals including Alkaloid, Flavonoid, Glycocides, Protein, Saponin, Steroid, Terpenoids and Tannin were tested according to standard method and confirmed [Harborne JB., 1998].

2.7. Antibacterial potential of plant extracts and antibiotics against Vibrio spp.

After being enriched for 48 h at 40 ◦C on tryptic soy broththe overnight V. parahaemolyticus culturewere spread out onto tryptic soy agar plates. Next, the inhibition zone (mm) was measured to assess the plant extracts (25, 50,75 mg/ml and 100 mg/ml) ability to inhibit V. parahaemolyticus. Additionally, a comparison between the findings of the antibiotic disks and the inhibition zone was conducted. The antibiotic disks included Tetracycline (TE) 30µg/disc, Amoxicillin (AX) 25µg/disc, Doxycycline (DO) 30µg/disc, Cefazolin (CZ) 30µg/disc, Gentamicin (GEN) 10µg/disc, Erythromycin (E) 15µg/disc (Solís-Soto, Prabhakarankutty et al., 2021).

2.8. Influence of combination between antibiotics and plant extracts against selected multi drug resistant strains

The most active methanolic plant extracts of *Tridax procumbens* L leaves was tested in combination with six antibiotics, two of the highest active antibiotics Tetracycline (TE) 30µg/disc, Amoxicillin (AX) 25µg/disc, Doxycycline (DO) 30µg/disc, Chloramphenicol (C)30µg/disc, Gentamicin (GEN) 10µg/disc, Erythromycin (E) 15µg/disc.Each antibiotic disk was loaded with 10µl of the extract.

3. Results and Discussion

3.1. Collection of Isoaltes

Twenty freshwater prawn culture samples were used in this study, and the results showed that *V. parahaemolyticus* was present in 14% of the samples while *V. vulnificus* was found in 6%. The results are presented in Table 3. Table 1 illustrates the biochemical confirmation of V. parahaemolyticus. A variety of studies have been carried out on different aquaculture farms. The study involved an examination of *P. monodon* culture ponds along the coast of Andhra Pradesh, leading to the isolation of six species of *Vibrios: V. harveyi, V. parahaemolyticus, V. alginolyticus, V. anguillarum, V. vulnificus, and V. splendidus*. Farm-produced feeds showed a notable occurrence of *V. parahaemolyticus, V. cholerae, E. coli, and Staphylococcus aureus* [Jayasree et al., 2006]. The three species *V. parahaemolyticus, V. cholerae, and V. vulnificus* are the most common bacterial diseases linked to seafood consumption worldwide, according to Maro et al. (2024). Additionally, *V. parahaemolyticus* was found in 25% of catfish samples compared to 22.6% of red tilapia fish samples, as reported by Noorlis et al. (2011). Globally, *V. parahaemolyticus* is the most common bacterial cause of gastroenteritis linked to seafood. According to Yang, Zhang et al. (2019), infections and outbreaks of *V. parahaemolyticus* have also steadily increased over the past 20 years.

Table1: Biochemical identification of pathogenic bacteria from fresh water prawn samples

S.No.	Test	Original colouroftthe	PositiveReaction	*V.parahaemolyticus*	*V.vulnificus*

		medium			
1	Urease	Organish yellow	Pink	+	+
2	Nitrate reduction	Colourless	Pinkishred	+	+
3	H_2Sproduction	Organish yellow	Black	-	-
4	Catalase	Colourless	Bubblesformation	+	+
5	VogesProskeur's	Colourless	Pinkishred	-	-
6	Methyl red	Colourless	Red	+	+
7	Indole	Colourless	Pinkishred	+	+
8	hemolysis	Red	β -hemolysis	+	+

Urease Test

Catalase Test

3.2. Phytochemical Screening of *Tridax procumbens*

After phytochemical test of *Tridax procumbens L leaves* methanol extract, the presence of alkaloids, flavonoids, glycocides, proteins, steroids and terpenoids were confirmed by visible observation of color change and chemical state change (Table 2).

Table 2: Determination of phyto-constituents of *Tridax procumbens* L. extract.

S.NO.	Phyto-constituents	Methanol extract
1	**Alkaloid**	+
2	**Flavonoid**	+
3	**Glycocides**	+
4	**Protein**	+
5	**Saponin**	-
6	**Steroid**	+
7	**Terpenoids**	+
8	**Tannin**	-

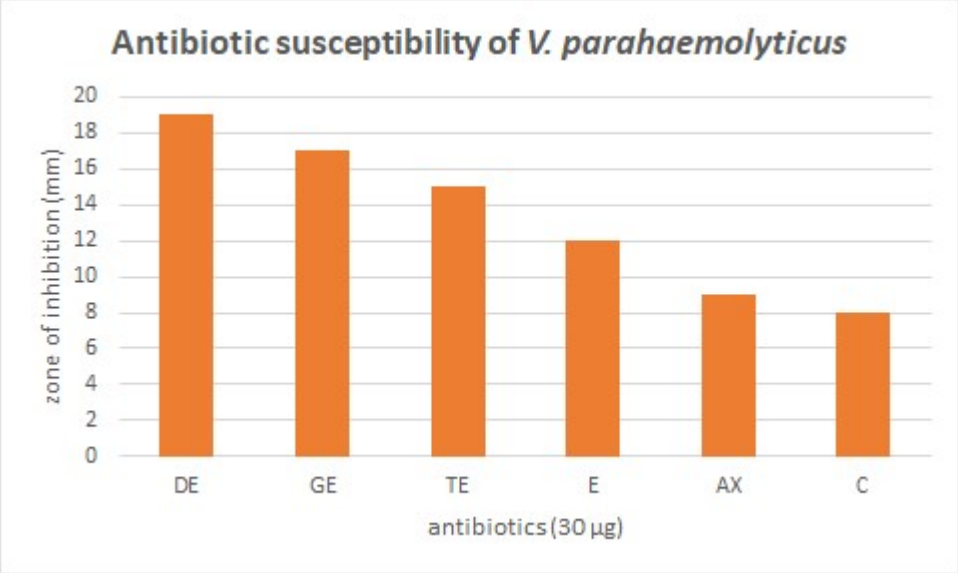
3.3. Antibiotic Susceptibility of *V. Parahaemolyticus*

This investigation examined the antibiotic susceptibility of the collected isolate to six antibiotics. The results presented in Table 3 indicate that the most effective antibiotics were Doxycycline, Gentamicin, and

Tetracycline, followed by Erythromycin, Amoxicillin, and Chloramphenicol, with inhibition zones measuring 19 mm, 17 mm, 15 mm, 12 mm, 9 mm, and 8 mm, respectively. Polyphenols can inhibit microorganisms, with their antibacterial activity being influenced by chemical structure and environmental factors (Almajano et al., 2007). T. procumbens L. methanolic extract's bioactive components were studied because they have numerous medical applications, including stopping the growth of bacteria and fungi and preventing infections in humans [Satya BS et al., 2012].

Table 3: Antibiotic susceptibility against

S.NO.	ANTIBIOTIC	SYMBOL	Conc. µg/disc	ZONE OF INHIBITION (mm)
1	DOXYCYCLIN	**DE**	**30 µg/disc**	**19**
2	GENTAMYCIN	**GE**	**10 µg/disc**	**17**
3	TETRACYCLINE	**TE**	**30 µg/disc**	**15**
4	ERYTHROMYCIN	**E**	**15 µg/disc**	**12**
5	AMOXYCILLIN	**AX**	**25 µg/disc**	**9**
6	CHLORAMPHENICOL	**C**	**30 µg/disc**	**8**



3.4. Antibacterial effect of Methanolic extract of *Tridax procumbens*

According to Koduru et al. (2007), a sizable portion of the world's population still primarily depends on medicinal plants for their healthcare, particularly in poor countries where the use of herbal medicine has a long and ongoing history. At 25, 50, 75, and 100 mg/ml, the methanolic extract of Tridax procumbens leaves showed strong antibacterial activity against V. parahaemolyticus. The inhibition zone measured between 10 and 24 mm, as assessed through the disc diffusion method. Table 2

Table 4: Antibacterial effect of Methanolic extract of *Tridax procumbens*:

S.NO.	Methanolic extract of *T.procumbens*	ZONE OF INHIBITION (mm)

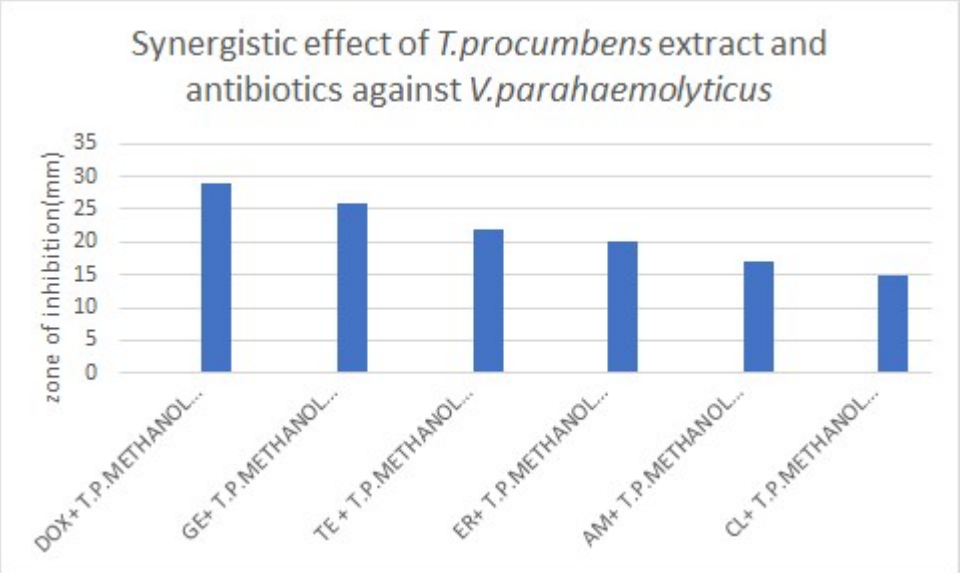
1	25 mg/ml	10
2	50 mg/ml	15
3	75 mg/ml	18
4	100 mg/ml	24

3.5. Influence of combination between antibiotics and plant extracts against *V. parahaemolyticus*

Although screening plant extracts for direct antimicrobial agents is a conventional approach, the alternative of identifying compounds that enhance resistance modulation and augment the efficacy of antibiotics in conjunction with plant extracts appeared more attractive. This strategy facilitates the reutilization of older, comparatively inexpensive antibiotics rendered ineffective by resistance development (Gibbons, 2004). The current study revealed that the amalgamation of specific antibiotics and Tridax procumbens leaf extract demonstrated considerable antibacterial efficacy against V. parahaemolyticus, as evaluated by the disc diffusion method.

Table 5: Synergistic effect of methanolic *Tridax procumbens* extract and Standard antibiotics against *V. parahaemolyticus*

S.NO.	ANTIBIOTIC + **Methanolic extract of***T.procumbens***(10 µg/ml)**	ZONE IF INHIBITION (mm)
1	DOXYCYCLIN	29
2	GENTAMYCIN	26
3	TETRACYCLINE	22
4	ERYTHROMYCIN	20
5	AMOXYCILLIN	17
6	CHLORAMPHENICOL	15



4. Conclusion

Even crude extracts of a number of plants showed good efficacy against *V. parahaemolyticus* bacterial strains,

which is noteworthy given the poor effect of modern antibiotic therapy. *Vibrio parahaemolyticus*, present in several prawn and fish products, is associated with septicaemia, wound infections, and gastroenteritis, which can manifest as abdominal pain and symptoms including diarrhoea, vomiting, fever, chills, and watery stools. Plant extracts, such as *Tridax procumbens,* are acknowledged for their variety of polyphenolic compounds. *Tridax procumbens* exhibits high concentrations of phenolic and flavonoid compounds, indicating significant antioxidant capabilities. The extract demonstrated improved antibacterial efficacy against *Vibrio parahaemolyticus,* as indicated by a greater inhibition zone diameter relative to synthetic antibiotics, and significantly reduced the growth of *Vibrio parahaemolyticus*. These plants may represent a potential source for the development of novel antibiotics to combat severe infections caused by *Vibrio spp.* bacteria. The interaction between antibiotics and plant extracts against resistant bacteria offers new possibilities for treating infectious diseases. When it stops working as a stand-alone treatment, this effect allows the use of the older and less costly antibiotic. Furthermore, the plant extracts might be a potential source of broad-spectrum compounds that change resistance, which could improve the effectiveness of antibiotics in treating infections caused by *Vibrio spp.*

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