International Journal of Biology Sciences

ISSN Print: 2664-9926
ISSN Online: 2664-9934
NAAS Rating (2026): 4.82
IJBS 2026; 8(1): 96-100
www.biologyjournal.net
Received: 15-11-2025
Accepted: 18-12-2025

Krina D Patel
Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India

Kailash H Vadher
Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Navsari, Gujarat, India

Rajkumar M Dabhi
Fisheries Research Station, Kamdhenu University, Sikka, Gujarat, India

Corresponding Author:
Krina D Patel
Department of Aquaculture, College of Fisheries Science, Kamdhenu University, Veraval, Gujarat, India

In vitro evaluation of the antibacterial activity of licorice (*Glycyrrhiza glabra*) root extract against *Vibrio parahaemolyticus*

Krina D Patel, Kailash H Vadher and Rajkumar M Dabhi

DOI: https://www.doi.org/10.33545/26649926.2026.v8.i1b.549

Abstract
The increasing prevalence of antibiotic resistance has intensified the search for alternative antimicrobial agents from natural sources. Licorice (*Glycyrrhiza glabra*) root is a well-known medicinal plant with diverse pharmacological properties. The present study aimed to evaluate the *in vitro* antibacterial activity of licorice root extract against *Vibrio parahaemolyticus* using the agar well diffusion method. 70% methanol was used as the negative control, while 0.01% chloramphenicol served as the positive control. All treatments were performed in triplicate to ensure reproducibility. The licorice root extract exhibited measurable zones of inhibition, indicating antibacterial potential, though its activity was lower compared to the standard antibiotic. No inhibitory effect was observed in the negative control, confirming the validity of the assay. The findings suggest that licorice root extract possesses promising antibacterial properties and may serve as a potential natural source of antimicrobial compounds. Further studies are recommended to isolate the active constituents and assess their mechanisms of action.

Keywords: Aquaculture. Antibiotics, extract and antimicrobial

Introduction
Aquaculture has emerged as one of the fastest-growing food production sectors worldwide; however, bacterial diseases remain a major constraint to its long-term sustainability (Ertor and Ortega-Cerda, 2019; Lin *et al*., 2018) [13, 22]. The widespread and prolonged use of synthetic antibiotics in aquaculture has raised serious concerns regarding antimicrobial resistance, environmental contamination, and the accumulation of drug residues in aquatic organisms and their products (Santos and Ramos, 2018; Devi *et al*., 2009) [28]. Consequently, increasing attention has been directed toward plant-derived bioactive compounds as eco-friendly and sustainable alternatives for disease management in aquaculture systems (Rattanavichai *et al*., 2015; Jesuniyi *et al*., 2020) [27, 20].

Glycyrrhiza glabra (commonly known as licorice) is a perennial leguminous plant whose roots are rich in diverse phytochemicals, including glycyrrhizin, flavonoids, saponins, coumarins, tannins, and sterols, many of which possess well-documented antimicrobial, antioxidant, and immunomodulatory properties (Tao *et al*., 2025; Hussain and Iqbal, 2019) [30, 19]. The genus Glycyrrhiza is derived from the Greek words glykos (sweet) and rhiza (root), reflecting the naturally sweet taste of its roots. Licorice is an herbaceous perennial plant belonging to the family Fabaceae and is native to West Asia, North Africa, and Southern Europe (Pastorino *et al*., 2018) [26]. Owing to its sweet and aromatic properties, licorice has been widely used as a flavoring agent in confectionery, tobacco, beverages, and pharmaceutical formulations, and is also marketed as a dietary supplement (Pastorino *et al*., 2018; Alagawany *et al*., 2019) [26, 3].

Licorice is highly valued in traditional and modern medicine for its broad range of health and nutritional benefits. The roots are particularly rich in glycyrrhizin, a major bioactive compound extensively used as a natural sweetener in food and tobacco products, which also enhances feed palatability in fish and other aquatic animals. Numerous studies have reported that G. glabra exhibits strong antioxidant, anti-inflammatory, hepatoprotective, anticoagulant, antifungal, and antibacterial activities, making it effective against various infectious diseases in fish (Hussain and Iqbal, 2019; Tao *et al*., 2025) [19, 30].

Licorice extracts are effective in inhibiting important aquaculture pathogens such as *Aeromonas hydrophila*, *Vibrio* spp., and *Staphylococcus aureus*, while simultaneously enhancing antioxidant defenses and immune responses in fish. Moreover, its natural origin minimizes the risk of antimicrobial resistance development compared to conventional synthetic antibiotics. Despite these promising attributes, challenges such as standardization of extraction methods, optimization of dosage, and regulatory approval for commercial application remain unresolved (Chen *et al*., 2024) [6].

Therefore, the present study was undertaken to perform phytochemical characterization and evaluate the antibacterial activity of *Glycyrrhiza glabra* root extracts, aiming to assess their potential as a sustainable and eco-friendly alternative for disease management in aquaculture.

Materials and Methods

Sample collection and preparation

The sample of *Glycyrrhiza glabra* (licorice) roots were collected from local herbal market and identified and confirmed by the botanist. Licorice roots were washed thoroughly with tap water and then after distilled water to remove impurities and dirt. The cleaned roots were shade-dried under room conditions until complete moisture removal and then pulverized into a fine powder using an electric grinder. The powdered root material was stored in airtight containers until extraction.

Extraction and dose preparation

Soxhlet extraction was employed for the isolation of bioactive compounds from the plant material following a modified method described by Da Castro & Priego Capte, (2008). Briefly, 40 g of the dried and powdered sample was accurately weighed and placed in a cellulose extraction thimble positioned in the Soxhlet apparatus. Methanol (400 ml) was used as the extraction solvent. The solvent was heated to reflux, allowing continuous condensation and percolation through the plant material. The extraction process was carried out for 6 h to ensure maximum recovery of bioactive constituents. After completion of extraction, the solvent was removed and the extract was concentrated to dryness using a steam evaporator.

The resulting dried crude extract was weighed and stored in sterile, properly labeled sample bottles until further analysis. The dried extract was re-dissolved in methanol and serially diluted to obtain working concentrations of 25, 50, 75, and 100 ppm for subsequent antibacterial activity assays.

For Negative control (NC) 70% methanol was used, while 0.01% chloramphenicol was served as positive control (PC). All experimental treatments were conducted in triplicate. Chloramphenicol was selected as the standard reference antibiotic to evaluate and compare the antibacterial efficacy of the licorice root extract.

Qualitative Phytochemical Analysis

The presence of major phytochemical constituents in the licorice root extract was qualitatively examined following standard protocols outlined by Harborne (1973) [16].

Flavonoids: 3 ml of extract were mixed with 1 mL of 10% sodium hydroxide solution. The formation of a yellow color that disappeared upon addition of dilute hydrochloric acid indicated the presence of flavonoids.

Tannins: 2 ml of extract were treated with 2-3 drops of 5% ferric chloride solution. The appearance of a blue-black or greenish coloration confirmed tannins.

Saponins: 5 ml of extract were vigorously shaken with an equal volume of distilled water. Persistent froth formation indicated saponins.

Glycosides: 5 ml of extract were hydrolyzed with 2.5 mL of 50% sulfuric acid and heated in a boiling water bath for 15 minutes. After cooling and neutralization with 10% sodium hydroxide, Fehling's solution was added and boiled. A brick-red precipitate indicated the presence of glycosides.

Alkaloids: 2 ml of extract were acidified with 10% hydrochloric acid and treated separately with Wagner's and Mayer's reagents. The formation of precipitates confirmed alkaloids.

Steroids: 2 ml of extract were dissolved in chloroform, and concentrated sulfuric acid was added gently. A reddish-brown ring indicated the presence of steroids.

Balsams: 9.5 ml of extract were mixed with an equal volume of 90% ethanol, followed by the addition of alcoholic ferric chloride solution. Greenish coloration indicated balsams.

Anthraquinones: 2 ml of extract were shaken with 10 mL of benzene and filtered. Five milliliters of 10% ammonia solution were added to the filtrate. The appearance of pink to violet coloration confirmed anthraquinones.

Volatile Oils: 1 ml of extract was treated with dilute hydrochloric acid. The absence of a white precipitate indicated the absence of volatile oils.

Bacterial Culture and Antibacterial Assay

The bacterial strain Vibrio parahaemolyticus was maintained on thiosulfate citrate bile salts sucrose (TCBS) agar medium. A loopful of actively growing culture was aseptically transferred and streaked onto TCBS agar plates, which were incubated at 37 °C for 24 h to obtain fresh bacterial growth.

Antibacterial activity of *Glycyrrhiza glabra* root extract was evaluated using the Kirby-Bauer disc diffusion technique. A standardized bacterial suspension was uniformly spread on Mueller-Hinton agar plates. Sterile paper discs (5 mm diameter) were impregnated with different concentrations of the licorice root extract (25-100 ppm) and placed on the inoculated agar surface. Discs containing 70% ethanol served as the negative control, while chloramphenicol (0.01%) was used as the positive control. The plates were incubated at 37 °C for 24 h.

Measurement of Inhibition Zone

Antibacterial activity was assessed by measuring the clear zones of inhibition around the discs. The diameter of the inhibition zones was recorded in millimeters using a Vernier caliper. Measurements were taken in two perpendicular directions, and the mean value was calculated to determine antibacterial potency.

Antibacterial Efficacy

The antibacterial efficacy of *Glycyrrhiza glabra* root extracts against V. parahaemolyticus was calculated according to Ghosh *et al*. (1997) [15] using the following formula:

$$\text{Antibacterial efficacy (\%)} = \frac{Mean\ inhibition\ zone\ of\ plant\ extract\ (mm)}{mean\ inhibition\ zone\ of\ antibiotic\ (mm)} \times 100$$

Results and Discussion

The qualitative phytochemical screening of the methanolic extract of *Glycyrrhiza glabra* roots revealed the presence of multiple secondary metabolites (Table 1). Positive results were observed for flavonoids, tannins, saponins, glycosides, steroids, and alkaloids, indicating a chemically diverse extract. However, anthraquinones, balsams, and volatile oils were not detected. The detected compounds are widely recognized for their biological activities and may collectively contribute to the antimicrobial potential of licorice roots.

Table 1: Qualitative phytochemical constituents of methanolic licorice root extract

Phytochemicals	Results
Flavonoids	+
Tannins	+
Saponins	+
Glycosides	+
Steroids	+
Alkaloids	+
Anthraquinones	-
Balsams	-
Volatile oils	-

(+ = Present), (- = Absent)

The antibacterial potential of the methanolic licorice root extract was assessed against *Vibrio parahaemolyticus* using the disc diffusion assay (Table 2). Clear zones of inhibition were formed around discs impregnated with the extract, confirming its antibacterial action. The negative control (methanol) showed no inhibitory effect, verifying that the solvent did not interfere with bacterial growth.

A gradual enhancement in inhibitory activity was noted with increasing extract concentration. The smallest inhibition zone was recorded at 25 ppm, whereas the largest zone was observed at 100 ppm. The standard antibiotic chloramphenicol produced the maximum inhibition, serving as a benchmark for comparison with the plant extract.

The relative antibacterial effectiveness of the licorice root extract increased proportionally with concentration (Table 2). Moderate antibacterial performance was observed at lower concentrations, while the highest concentration (100 ppm) exhibited strong inhibitory activity, achieving approximately half of the effectiveness of the standard antibiotic. These results demonstrate a clear dose-dependent response and highlight the potential of *Glycyrrhiza glabra root* extract as a natural antibacterial agent against *V. parahaemolyticus*.

Table 2: Antibacterial activity and relative effectiveness of *Glycyrrhiza glabra* (licorice) root extract against *Vibrio parahaemolyticus*

Treatment	Sample	Dose	Inhibition zone (mm) ± SE	Relative efficacy (%)	Activity level
PC	Chloramphenicol	0.01%	23.4 ± 0.4	100	Very strong
NC	Ethanol	70%	0.0 ± 0.0	0	No activity
T_1	Licorice root extract	25 ppm	4.2 ± 0.2	18.0	Moderate
T_2	Licorice root extract	50 ppm	6.1 ± 0.3	26.1	Moderate
T_3	Licorice root extract	75 ppm	8.7 ± 0.3	37.2	Moderate
T_4	Licorice root extract	100 ppm	11.9 ± 0.4	50.9	Strong

This study found that *Glycyrrhiza glabra* roots have a rich phytochemical profile and show antibacterial activity against *Vibrio parahaemolyticus*. Qualitative phytochemical screening showed that the methanolic licorice extract contains flavonoids, tannins, saponins, glycosides, steroids, and alkaloids. These results align with earlier studies describing licorice as a medicinal plant with a wide range of chemical constituents, including several groups of secondary metabolites that show biological activity (Abdellatif *et al*., 2023) [1]. Because of anthraquinones, balsams, and volatile oils are not present, the antimicrobial activity of licorice likely comes mainly from non-volatile polyphenolic and glycosidic compounds (Karahan *et al*., 2024) [21].

The antibacterial assay showed that licorice root extract inhibited *V. parahaemolyticus* in a dose-dependent manner, with stronger inhibition observed at higher concentrations. As the extract concentration increased, the inhibition zone diameter and relative efficacy also increased, which is consistent with a dose-response relationship. Chloramphenicol showed higher antibacterial activity than the licorice extract, while the licorice extract produced moderate to strong inhibition only at the highest concentrations. Comparable trends have been reported in earlier studies, where plant extracts showed weaker antibacterial activity than synthetic antibiotics but still produced biologically meaningful effects (Doughari, 2012; Cowan, 1999) [12, 7].

The antimicrobial efficacy of *G. glabra* can be largely attributed to the synergistic interaction of its phytochemical constituents. Flavonoids are widely recognized for their ability to disrupt bacterial cell membranes, inhibit energy metabolism, and interfere with nucleic acid synthesis (Cushnie & Lamb, 2011) [9]. Tannins can limit microbial activity by binding to microbial proteins, enzymes, and cell wall components, which can slow bacterial growth and disrupt metabolism (Scalbert, 1991) [29]. Saponins can increase membrane permeability, which may cause intracellular contents to leak and can lead to cell lysis (Francis *et al*., 2002) [14], whereas alkaloids limit bacterial growth by disrupting DNA replication and protein synthesis (Cushnie *et al*., 2014) [8]. Combined effect of these compounds may account for the antibacterial activity observed against *Vibrio parahaemolyticus*.

Due to absence of volatile oils, the antimicrobial activity seen in this study is likely not due to essential oil constituents, rather than more too stable polyphenolic and glycosidic compounds. This approach is well suited to aquaculture and food systems, where the volatility and instability of essential oils can restrict their use in practice (Burt, 2004) [4]. Previous studies report that extracting licorice with methanol yields a higher amount of phenolic compounds than water-based extraction, and the methanolic extract shows stronger antimicrobial activity than aqueous extracts ((Ahmed *et al*., 2022; Harborne, 1998) [2, 17].

Comparisons with previous studies further support the findings of the present work. Abdellatif *et al*., (2023) [1] reported strong antibacterial activity of *G. glabra* extracts against oral and foodborne pathogens, while Karahan *et al*., (2024) [21] demonstrated significant antioxidant and antimicrobial properties across different *Glycyrrhiza* species. Additionally, Ahmed *et al*. (2022) [2] observed that methanolic licorice extracts exhibited broader and stronger antimicrobial activity than water-based extracts, which aligns with the solvent choice and results of the present study. The effectiveness of licorice against Gram-negative bacteria such as *Vibrio* spp. is particularly important, as these organisms are often more resistant due to their outer membrane barrier (Nikaido, 2003; Charan *et al*., 2022) [25, 5].

From an applied standpoint, these results suggest that licorice root extract could be used as a natural antimicrobial agent in aquaculture. Rising concerns about antibiotic resistance, environmental pollution, and drug residues have led researchers to consider plant-based options that break down in the environment and may be less likely to drive resistance (Ventola, 2015; Newman & Cragg, 2020) [31, 24]. Licorice roots phytochemicals act on more than one target, which lowers the chance that resistance will develop and supports their inclusion in integrated disease management strategies.

These results are encouraging, but the study has limitations that should be noted. This study was limited to *in vitro* testing, so *in vivo* studies are still needed to confirm the antibacterial activity, bioavailability, and safety of licorice extracts under normal physiological conditions (Namdeo, 2021) [23]. Future research should isolate and identify individual active compounds and test how they act together with standard antibiotics, including possible synergistic interactions. These combination therapies can improve antibacterial activity and may allow lower antibiotic doses (Hemaiswarya *et al*., 2008) [18].

Conclusion

From the findings of the current study, it is revealed that the antibacterial activity of the licorice root methanolic extract (*Glycyrrhiza glabra*) against *V. parahaemolyticus* when compared to the commonly used antibiotic chloramphenicol is significant. Though the inhibitory potential of the licorice root extract was low compared to the positive control, the zones of inhibition determined its potential to be a promising antibacterial agent. The lack of zone of inhibition in the case of the negative control also proves the reliability of the experiment. The above findings also revealed that the licorice root extract may act as a potential agent to produce effective bioactive compounds to develop alternative or complementary antibacterial agents.

Acknowledgements: The author expresses gratitude to College of Fisheries Science, Kamdhenu University, Veraval and National Fellowship for ST Fellow scheme (NFST) for financial support and for allowing the smooth conduction of the experiments. Special thanks to Dr. K. H. Vadher for guidance and inspiration during this research work.

Ethical statement: This study involved only *in vitro* experiments and did not require ethical approval.

References

1. Abdellatif AO, Ahmed HA, Osman OM, *et al*. Evaluation of the antimicrobial activity of licorice (*Glycyrrhiza glabra*) plant against representative oral pathogens. Journal of Applied Health Sciences and Medicine. 2023;3(6):17-27.
2. Ahmed S, Ahmad A, Izhar K, Safdar MN, Raja MU, Aslam I. Effect of different solvents on phytochemical and antimicrobial potential of licorice extract. World Journal of Biology and Biotechnology. 2022;7(1):7-12.
3. Alagawany M, Elnesr SS, Farag MR, Abd El-Hack ME, Khafaga AF, Taha AE, Dhama K. Use of licorice (*Glycyrrhiza glabra*) herb as a feed additive in poultry: current knowledge and prospects. Animals. 2019;9(8):536.
4. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology. 2004;94(3):223-253.
5. Charan S, Nirupama U, Krishna MC, Kumar DTM, Manasa B, Varsha J, Babu MK. Phytochemical screening and medicinal activities of *Glycyrrhiza glabra*. World Journal of Pharmacy and Pharmaceutical Sciences. 2022;11(12):1750-1780.
6. Chen RY, Shi JJ, Liu YJ, Yu J, Li CY, Tao F, *et al*. The state-of-the-art antibacterial activities of glycyrrhizin: a comprehensive review. Microorganisms. 2024;12(6):1155.
7. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999;12(4):564-582.
8. Cushnie TPT, Cushnie B, Lamb AJ. Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities. International Journal of Antimicrobial Agents. 2014;44(5):377-386.
9. Cushnie TT, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. International Journal of Antimicrobial Agents. 2011;38(2):99-107.

10. De Castro ML, Priego-Capote F. Soxhlet extraction: past and present panacea. Journal of Chromatography A. 2010;1217(16):2383-2389.
11. Dewi NR, Huang HT, Wu YS, Liao ZH, Lin YJ, Lee PT, Nan FH. Guava (*Psidium guajava*) leaf extract enhances immunity, growth, and resistance against *Vibrio parahaemolyticus* in white shrimp *Penaeus vannamei*. Fish & Shellfish Immunology. 2021;118:1-10.
12. Doughari JH. Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents. Rijeka: INTECH Open Access Publisher; 2012. p. 1-33.
13. Ertor I, Ortega-Cerdà M. The expansion of intensive marine aquaculture in Turkey: the next-to-last commodity frontier? Journal of Agrarian Change. 2019;19(2):337-360.
14. Francis G, Kerem Z, Makkar HPS, Becker K. The biological action of saponins in animal systems: a review. British Journal of Nutrition. 2002;88(6):587-605.
15. Ghosh A, Das BK, Roy A, Mandal B, Chandra G. Antibacterial activity of some medicinal plants. Journal of Natural Medicines. 1997;62:259-262.
16. Harborne JB. Phenolic compounds. In: Phytochemical methods: a guide to modern techniques of plant analysis. Dordrecht: Springer Netherlands; 1973. p. 33-88.
17. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 3rd ed. Dordrecht: Springer Science & Business Media; 1998.
18. Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. Phytomedicine. 2008;15(8):639-652.
19. Hussain F, Iqbal I. Nutritional and therapeutic potential of *Glycyrrhiza glabra* L. roots. Asian Journal of Medical and Biological Research. 2019;5(4):265-270.
20. Jesuniyi IF, Moruf RO, Lawal-Are AO. Immunomodulatory effect of *Moringa oleifera* Lam. aqueous extract on the burrowing crab *Cardiosoma guanhumi* (Latreille, 1828). Nigerian Veterinary Journal. 2020;41(3):264-273.
21. Karahan F, Turkmen M, Avşar C, Gezici S. Phytochemical profiling and *in vitro* antioxidant, antimicrobial, cytotoxicity effects of some *Glycyrrhiza* species from Turkey. Pharmaceutical Chemistry Journal. 2024;58(2):99-111.
22. Lin SY, Lee CH, Huang ES, Sheu SC, Yu HS. Quantification of crustacean tropomyosin, a major food allergen, in eight species of Taiwanese shrimp based on immunoassay. Food Analytical Methods. 2018;11(9):2607-2613.
23. Namdeo P. Phytochemistry and pharmacological studies of *Glycyrrhiza glabra*: a medicinal plant review. International Journal of Pharmaceutical Sciences Review and Research. 2021;67(1):30-38.
24. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 1981 to 2019. Journal of Natural Products. 2020;83(3):770-803.
25. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiology and Molecular Biology Reviews. 2003;67(4):593-656.
26. Pastorino G, Cornara L, Soares S, Rodrigues F, Oliveira MBP. Liquorice (*Glycyrrhiza glabra*): a phytochemical and pharmacological review. Phytotherapy Research. 2018;32(12):2323-2339.
27. Rattanavichai W, Chen YN, Chang CC, Cheng W. The effect of banana (*Musa acuminata*) peels hot-water extract on immunity and resistance of giant freshwater prawn *Macrobrachium rosenbergii* via dietary administration. Fish & Shellfish Immunology. 2015;46(2):378-386.
28. Santos L, Ramos F. Antimicrobial resistance in aquaculture: current knowledge and alternatives to tackle the problem. International Journal of Antimicrobial Agents. 2018;52(2):135-143.
29. Scalbert A. Antimicrobial properties of tannins. Phytochemistry. 1991;30(12):3875-3883.
30. Tao F, Cao JF, Liu YJ, Chen RY, Shi JJ, Li CY, *et al*. Evaluation on the antibacterial activity of glycyrrhizin against *Pseudomonas plecoglossicida* in ayu fish (*Plecoglossus altivelis*). Aquaculture. 2025;595:741520.
31. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and Therapeutics. 2015;40(4):277-283.