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Antimicrobial Activity of Leaf, Stem and Root Extracts of *Hemidesmus indicus* Against Aquatic Pathogens

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Abstract: The present study aimed to assess the antimicrobial activity of *Hemidesmus indicus* against aquatic pathogens. *Hemidesmus indicus* is a twining shrub with lactiferous branches that produces numerous bioactive compounds. Plant parts are commonly used in traditional medicine to treat a variety of diseases. A comparative evaluation of the antimicrobial activity of Hexane, Ethyl acetate, Ethanol and water extracts of leaf, stem ad root of the *Hemidesmus indicus* was tested against the aquatic pathogens including bacteria and fungi by standard agar well diffusion method. The results indicated that all the plant parts exhibited varying degrees of antimicrobial properties. However, among the selected parts the highest antimicrobial property was shown by root against the bacteria *Streptococcus agalactiae* (16 mm), *Streptococcus iniae* (12 mm), *Aeromonas hydrophila* (20 mm), *Vibrio cholera* (21 mm), *Pseudomonas aeruginosa* (19 mm), and the fungus *Aspergillus niger* (19 mm) and *Candida albicans* (15 mm). Whereas the ethyl acetate fractions of the leaf showed maximum inhibitory activity against aquatic pathogens next to the root and the aqueous extract of the stem exhibited strong inhibition against *Vibrio cholera*. The study confirmed that the ethyl acetate extract of the *Hemidesmus indicus* root has high antibacterial activity against fish pathogens meanwhile the ethanol extract exhibited high antifungal activity. The present investigation confirmed the efficacy of *Hemidesmus indicus* as a natural alternative to synthetic antibiotics making promising contributions to the field of drug discovery and tremendous scope in the field of aquaculture.

Keywords: Antimicrobial Activity, Hemidesmus indicus, Aquatic pathogens, Agar well diffusion, Fish, Antifungal, Antibacterial

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Introduction

Infectious microbes are major threats to fish farming and the indiscriminate use of synthetic antibiotics may promote undesirable side effects concerning aquaculture sustainability (Dawood *et al*., 2021). Drug resistance is a major problem that has evolved from the overuse of antibiotics in the field of aquaculture (Jordi *et al*., 2019). The use of eco-friendly alternatives to control infectious

diseases in fish has increased the elimination of drug-resistant bacteria in aquaculture (Harnisz *et al*., 2010). The demand for antibiotics with high potency and low toxicity has increased dramatically, and biodegradable natural antibiotics have been proposed as alternatives to chemical antibiotics (Srichaiyo *et al*., 2020). World Health Organization states that 80% of the world's population uses various plant fractions and their dynamic constituents as traditional medicines (Silva *et al*., 2017). The therapeutic usefulness of plants is determined by their chemical contents or phytochemical ingredients, which are naturally present in plants (Deepa *et al*., 2012). Medicinal plants contain active bio-constituents such as flavonoids, alkaloids, tannins, saponins, phenols, and glycosides responsible for antioxidant, anti-inflammatory, anticancer, and antimicrobial properties (Pan *et al*., 2011). It is essential to study medicinal plants in order to promote their proper use and determine their potential as sources of new drugs. *Hemidesmus indicus* (L.) R. Br. ex Schult. (Asclepiadaceae), is extensively used in traditional medicine throughout the Indian subcontinent due to the numerous biological activities attributed to its various parts, particularly the roots (Das and Bisht, 2012). It has been used for centuries to treat snakebites, scorpion stings, diabetes, urinary diseases, dyspnea, menorrhagia, oligospermia, anorexia, fever, abdominal colic and pain, dysentery, diarrhoea, cough, rheumatism, headache, inflammation, pyrosis, skin diseases, leprosy, sexually transmitted diseases, and cancer (Samapika Nandya *et al*., 2020). It contains saponins, tannins, hemidesmine, hemidesmol, hemidesterol, stearoptin, pregnane glycosides, -sitosterol, coumarin, volatile oils, triterpenes, flavonoids, and so on (Moorthy and Kumar, 2021). *H. indicus* has been suggested to have antimicrobial therapeutic potential against fish infectious diseases. To the best of our knowledge, there exists no study regarding the antimicrobial activity of *H. indicus* against pathogens in fish. In general, different parts of plants possess a variety of constituents that contribute to a variety of biological activities. Therefore we compared the antimicrobial activities of different parts of the *H. indicus* for paving the way for the development of natural antibiotics against harmful microbes.

Materials and Methods

Collection and authentication of plant material:

Hemidesmus indicus (L.) R. Br. Plants were collected from Palakkad district, Kerala, India. The plant was authenticated from Botanical Survey of India, Coimbatore (Ref No. BSI/SRC/5/23/2022/TECH/410).

Preparation of plant extracts:

The collected plant material was washed thoroughly 3-4 times under running tap water and then separated the plant parts as leaves, stems and roots and then allowed to dry at room temperature. The dried parts were crushed into fine powder by using an electric blender and stored in separate airtight polyethene bags. About 50 g of each powdered plant material was extracted in soxhlet apparatus (Borosil Glass Workers Ltd, Mumbai, India) for 72 h at 55-60 °C, using lipid-soluble polar solvents (hexane, ethyl acetate, ethanol, and water). The obtained filtrate was concentrated by rotary evaporation at 40 °C and kept in the refrigerator at 4°C in air-tight bottles for future analysis.

Collection of microbial culture:

The antibacterial activity of the plant was tested against five aquatic pathogenic bacteria which include two Gram-positive strains such as *Streptococcus agalactiae* ATCC13813, and *Streptococcus iniae* ATCC29178, and three Gram-negative strains such as *Aeromonas hydrophila* MTCC12301, *Vibrio cholera* MTCC 3906 and *Pseudomonas aeruginosa* MTCC741. All the bacteria were obtained from Microbiology Laboratory at CMFRI, Cochin. The pathogenic fungi tested were *Aspergillus niger* ATCC281 and *Candida albicans* ATCC10231 and were obtained from TRI-Biotech, Trichy, India.

Antimicrobial activity by agar well diffusion Method:

Screening Antibacterial activity:

Antibacterial activity of the crude extracts of leaf, stem and root was done by standard agar well diffusion method with slight modifications. The bacterial culture medium was made from nutrient agar by dissolving 28 g in 1000 ml of distilled water and then autoclaved at 15 lbs pressure and 121°C for 15 min. After removing it from the autoclave, it was immediately cooled to 50-55°C in a water bath. The nutrient agar medium was poured into sterile Petri dishes to a uniform depth of 4 mm, which is about 25 ml in a 90 mm plate allowed to solidify. After solidification 1 ml of the bacterial suspension (*Streptococcus agalactiae*, *Streptococcus iniae*, *Aeromonas hydrophila*, *Vibrio cholera* and *Pseudomonas aeruginosa*) were swabbed by using a sterile cotton swab to ensure an even distribution of the inoculums. The plates were left undisturbed for 3 to 5 min to allow excess moisture to absorb. By using a sterile cork borer 4 wells were made and poured the samples with different concentrations (25 µl, 50 µl, 75 µl), and amoxiclav (10 mcg) as a control. The culture media was incubated at 37^0 C for 24 h keeping the Petri dishes invertedly. The diameter of the inhibition zone was measured in millimetres (Magaldi *et al*., 2004).

Screening antifungal activity:

The fungal culture was inoculated on potato dextrose agar medium, made by dissolving 40 g potato infusion, 4 g dextrose, and 3.5 g agar in 200 ml of distilled water. The prepared medium was autoclaved for 15 min at 15 lbs at 121 °C. The autoclaved medium was mixed thoroughly and poured into 100 mm Petri plates (25-30 ml/plate). The anti-fungal agent in the given sample was allowed to diffuse into the medium and interact with the test organisms in a freshly seeded plate. As a result of the mycelial growth, the resulting zones of inhibition were uniformly circular. The diameter of the zone of inhibition was measured in millimetres. Petri plates having 20 ml potato dextrose agar medium were seeded with a 72 h culture of fungal strains (*Aspergillus niger* and *Candida albicans*). Into the wells samples of various concentrations (50, 100, 250, and 500 ml) and Amphotericin (100 units) as positive control were added. Inoculated media were incubated for 48-72 h at 37 °C. The diameter of the inhibition zone was measured in millimetres. The values were calculated using Graph Pad Prism 6.0 software (USA).

Results

The antimicrobial activity of the *Hemidesmus indicus* plant was determined by the agar well diffusion method. Fish pathogenic microbes such as *S. agalactiae*, *S. iniae*, *A. hydrophila*, *P. aeruginosa*, *V. cholera*, *A. niger* and *C. albicans* were selected and the antimicrobial activity was assessed by measuring the diameter of the inhibition zone formed around the well containing an extract from different part of *H. indicus* at varying concentrations were analysed.

The diameter of inhibition zones was measured in millimetres as given in Tables 1, 2, 3 and 4. The diameter of inhibition zones for various extracts of *H. indicus* leaf is given in Table 1. Ethyl acetate extract has high antimicrobial activity against 4 bacteria. However, Hexane, ethanol and aqueous extracts of *H. indicus* leaf have antimicrobial activity against 3 bacteria. Ethyl acetate extract has high antibacterial activity (Table 1) against *S. agalactiae* (19 mm) at 50 µl sample. However, Hexane extract of the leaf has high antibacterial activity against *S. agalactiae* (17 mm) and *S. iniae* (17 mm) whereas ethanol extract has a maximum zone of inhibition against *A. hydrophila* (14 mm) whereas aqueous extract has high antimicrobial activity against *S. agalactiae* (17 mm). Furthermore aqueous extract has high antifungal activity (Table 4) against *A. niger* and all other three extracts have the same level of inhibition against *A. niger* (16,13,13 and13 mm, respectively) at 500 µl sample. Ethyl acetate, ethanol, hexane and aqueous extracts showed high antifungal activity against *C. albicans* (15, 13, 11 and 9 mm, respectively) at 500 µl sample. These results demonstrated that the Ethyl acetate extract of *H. indicus* leaf has high antimicrobial activity against fish pathogens.

Table 1: Anti-bacterial activity of *Hemidesmus indicus* leaf

ZONE OF INHIBITION						
EXTRACT	Conc. (µl)	*S. agalactiae*	*S. iniae*	*A. hydrophila*	*P. aeruginosa*	*V. cholera*
Hexane	25 µl	11 ± 0.05*	0	11 ± 0.17*	0	0
	50 µl	12 ±0.11*	0	12 ± 0.05*	0	0
	75 µl	17 ± 0.51*	17 ± 0.51*	9 ± 0.05*	0	0
	Disc	21 ± 0.63	20 ± 0.11	17 ± 0.51	21 ± 0.75	10 ±0.05
Ethyl acetate	25 µl	11 ± 0.17*	0	12 ± 0.05*	0	0
	50 µl	19 ± 0.05*	0	14 ± 0.23*	0	0
	75 µl	10 ± 0.05*	12 ± 0.11*	16 ± 0.63*	0	12 ± 0.11*
	Disc	21 ± 0.69	14 ± .23	20 ± 0.64	21 ± 0.75	15 ± 0.05
Ethanol	25 µl	0	0	9 ± 0.05*	10 ± 0.05*	0
	50 µl	13 ± 0.11*	0	14 ± 0.23*	11 ± 0.17*	0
	75 µl	11 ± 0.17*	0	13 ± 0.11*	13 ± 0.11*	0
	Disc	20 ± 0.11	13 ± 0.11	17 ± 0.51	20 ± 0.64	19 ± 0.05
Aqueous	25 µl	11 ± 0.17*	0	9 ± 0.05*	9 ± 0.05*	0
	50 µl	13 ± 0.11*	0	10 ± 0.05*	10 ± 0.05*	0
	75 µl	17 ± 0.51*	0	12 ± 0.28*	13 ± 0.11*	0
	Disc	0	20 ± 0.11	16 ± 0.63	18 ± 0.34	21 ± 0.75

Values are in Mean ± SE, where n = 3, each with triplicate samples. Statistical comparison was made with the positive control group; ** $p < 0.01$.

The diameter of inhibition zones for stem extracts of *H. indicus* is given in Tables 2 and 4. Ethanol extract of the stem has high antimicrobial activity against 4 bacteria and ethyl acetate extract has antibacterial activity against 3 bacteria; however, hexane extracts have inhibition against 2 and aqueous extract has antimicrobial activity against only 1 bacteria. The aqueous extract of the stem has high antibacterial activity against *V. cholera* (18 mm) at 75 µl sample (Table 2), however, ethyl acetate extract has high activity against *V. cholera* (17mm) at 50 and 75 µl, respectively. Hexane extract exhibited its maximum activity against *S. iniae* (14mm) and ethanol extract has its maximum inhibitory activity against *S. iniae* and *A. hydrophila* at 75 µl sample. Ethanol, ethyl acetate, aqueous and hexane extracts have high antifungal activity against *A. niger* (18, 17, 15 and 14 mm,

Table 2: Anti-bacterial effect of *Hemidesmus indicus* stem

ZONE OF INHIBITION						
EXTRACT	Conc. (µl)	*S. agalactiae*	*S. iniae*	*A. hydrophila*	*P. aeruginosa*	*V. cholera*
Hexane	25 µl	0	9±0.05*	0	0	0
	50 µl	0	11±0.05*	0	0	0
	75 µl	9±0.05*	14±.1.09*	0	0	0
	Disc	17±0.05	19±0.05	19±0.05	16±0.63	16±0.63
Ethyl acetate	25 µl	0	0	11±0.17*	0	0
	50 µl	0	0	11±0.17*	0	17±0.51*
	75 µl	11±0.17*	0	13±0.11*	0	17±0.05*
	Disc	20±0.64	19±0.05	20±0.64	21±0.63	21±0.75
Ethanol	25 µl	9±0.05*	9±0.05*	0	0	0
	50 µl	10±0.05*	9±0.05*	0	0	0
	75 µl	10±0.05*	12±0.17*	12±0.05*	0	9±0.05*
	Disc	20±0.64	17±0.51	16±0.63	20±0.64	21±0.69
Aqueous	25 µl	0	0	0	0	15±.05*
	50 µl	0	0	0	0	16±.63*
	75 µl	0	0	0	0	18±1.05*
	Disc	20±0.64	19±0.05	21±0.69	17±0.51	17±0.51

Values are in Mean ± SE, where n = 3, each with triplicate samples. Statistical comparison was made with the positive control group; * $p < 0.01$.

respectively) at 500 µl sample (Table 4). However, ethyl acetate, ethanol, hexane and aqueous extracts showed high antifungal activity against *C. albicans* (15, 12, 10 and 10 mm, respectively) in 500 µl samples. From these results, it is concluded that the aqueous extract of the *H. indicus* stem is more powerful against *V. cholera* infection.

The antimicrobial activity of root extracts of *Hemidesmus indicus* is shown in Table 3. Hexane and ethyl acetate extract have antibacterial activity against 5 bacteria; aqueous extract has antibacterial activity against 4 bacteria and ethanol extract has antibacterial activity against 3 bacteria. The ethyl acetate extract of the root has high antibacterial activity against *A. hydrophila* (20 mm) at 75 µl sample (Table 3), however, the aqueous extract has high activity against *P. aeruginosa* (19 mm) at 75 µl sample whereas

Table 3: Anti-bacterial effect of *Hemidesmus indicus* root

ZONE OF INHIBITION						
EXTRACT	Conc. (µl)	*S. agalactiae*	*S. iniae*	*A. hydrophila*	*P. aeruginosa*	*V. cholera*
Hexane	25 µl	13±0.11*	9±0.51*	0	12±0.01*	9±0.05*
	50 µl	14±1.09*	12±0.11*	9±0.05*	13±0.11*	12±0.17*
	75 µl	16±0.63*	13±0.11*	9±0.05*	15±0.05*	15±0.05*
	Disc	17±0.51	21±0.75	17±0.51	22±1.73	21±0.75
Ethyl acetate	25 µl	0	9±0.05*	12±0.11*	11±0.17*	16±0.63*
	50 µl	10±0.05*	13±0.11*	19±0.05*	17±0.51*	19±0.05*
	75 µl	15±0.05*	19±0.05*	20±0.64*	19±0.05*	21±0.75*
	Disc	16±0.63	21±0.75	22±.1.73	19±0.05	23±1.15
Ethanol	25 µl	0	10±0.05*	0	0	0
	50 µl	0	16±0.63*	0	0	0
	75 µl	0	16±.63*	0	0	12±0.11*
	Disc	17±0.05	21±.69*	19±0.05	19±0.05	21±0.75
Aqueous	25 µl	9±0.05*	9±0.05*	0	0	0
	50 µl	10±0.05*	11±0.17*	0	15±0.05*	9±0.05*
	75 µl	12±0.05*	11±0.17*	0	17±0.51*	13±0.11*
	Disc	21±0.63	20±0.64	19±0.05	18±1.05	20±0.64

Values are in Mean ± SE, where n = 3, each with triplicate samples. Statistical comparison was made with the positive control group; * $p < 0.01$.

hexane extract exhibited its maximum activity against *S. agalactiae* (16 mm) and ethanol extract has its maximum inhibitory activity against *S. iniae* (12 mm) at 50 and 75 µl sample. Ethyl acetate extract has maximum inhibition against *V. cholera* (21 mm) at 75 µl sample. Ethanol, ethyl acetate, aqueous and hexane extracts have high antifungal activity against *Aspergillus niger* (19, 18, 15 and 14 mm, respectively) at 500 µl sample (Table 4). Ethanol, ethyl acetate, aqueous and hexane extracts showed high antifungal activity against *Candida albicans* (15, 13, 11 and 10 mm, respectively) at 500 µl sample. These results demonstrated that ethyl acetate extract of the *Hemidesmus indicus* has high antibacterial activity against fish pathogens whereas ethanol extract exhibited high antifungal activity against *Aspergillus niger*.

Table 4: Anti-fungal activity of *Hemidesmus indicus* leaf, stem and root

EXTRACT	CONC. (µl)	LEAF		STEM		ROOT	
		A. niger	*C. albicans*	*A. niger*	*C. albicans*	*A. niger*	*C. albicans*
Hexane	50	0	0	0	0	8±0.57*	8±0.05*
	100	0	0	0	0	9±0.05*	9±0.05*
	250	0	8±0.04*	10±0.05*	9±0.05*	10±0.05*	9±0.05*
	500	13±0.11*	9±0.05*	14±0.09*	10±0.05*	14±0.09*	10±0.05*
	CONTROL	15±0.05	13±0.11	15±.05	13±0.11	15±0.05	13±0.53
Ethyl acetate	50	0	0	0	0	9±0.05*	8±0.57*
	100	0	0	0	0	10±0.05*	9±0.05*
	250	0	13±0.11*	14±1.09*	0	11±0.17*	10±0.05*
	500	13±0.05*	15±0.05*	17±0.51*	15±0.05*	18±0.05*	13±0.05*
	CONTROL	16±0.51	16±0.63	19±0.05	16±0.63	17±0.51	11±0.17
Ethanol	50	0	0	0	0	11±0.17*	9±0.05*
	100	0	0	0	0	13±0.11*	10±0.05*
	250	0	11±0.17*	15±0.05*	9±0.05*	15±0.05*	11±0.17*
	500	13±0.11*	13±0.11*	18±0.05*	12±0.17*	19±0.05*	15±0.05*
	CONTROL	15±0.63	15±0.05	19±0.05	15±0.05	16±0.17	14±1.09
Aqueous	50	0	0	11±0.17*	0	9±0.11*	8±0.57*
	100	11±0.17*	0	12±0.05*	0	9±0.05*	8±0.57*
	250	13±0.11*	0	12±0.11*	0	10±0.63*	9±0.57*
	500	16±0.63*	11±0.05*	11±0.05*	10±0.05*	11±0.05*	10±0.05*
	CONTROL	17±0.53	15±0.05	17±0.51	15±0.05	17±0.05	15±0.05

Values are in Mean ± SE, where n = 3, each with triplicate samples. Statistical comparison was made with the positive control group; * $p < 0.01$.

From this study, we have found that antimicrobial activity differed substantially depending on the plant part and microbes tested. Based on the results *H. indicus* root showed high antimicrobial activity against the tested fish pathogens however, the leaf extracts showed moderate antimicrobial activity whereas stem extracts of *H. indicus* showed only a weak mode of activity against tested pathogens.

Discussion

Many researchers worldwide have looked into medicinal plants' antimicrobial properties and their active compounds. Herb extracts have strong antibacterial properties against both Gram-positive and Gram-negative bacteria. Several compounds derived from medicinal plants inhibited Gram-positive bacterial growth and can even be used against viruses, parasitic, and fungal diseases (Harikrishnan *et al*., 2009). *Hemidesmus indicus* has numerous medicinal properties varying from chemo-preventive, anti-diarrheal, anti-cancerous, wound healing power, immuno-modulatory, antioxidant, anti-venoms, anti-leprotic, diuretic properties etc. (Kotteswari *et al*., 2018). Previous studies reported *in vitro* antibacterial activity of *H. indicus* n-butanolic extract's inhibitory activity against human pathogenic bacterial strains viz. *B. substilis*, *E. coli*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Staphylococcus aureus* (Riazunnisa *et al*., 2013). Another study found that the bioactive compound

2-Hydroxy-4-methoxy benzaldehyde has antibacterial and antifungal activities and exhibited a large degree of inhibition against *Staphylococcus aureus* (Mehmood *et al*., 2016). The active constituent saponin from the *H. indicus* roots extract showed antimicrobial activity against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhi*, *Aspergillus flavus*, *A. niger* and *A. fumigatus* (Khanna and Kannabiran, 2008). Gayathri and Kannabiran (2009) reported that aqueous extracts of *H. indicus*, *Ficus bengalensis* and *Pterocarpus marsupium* have antimicrobial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia*. Another study found that with a concentration of 150 mg/ml/well, ethanolic extract showed significant antibacterial activity against six multidrug-resistant bacteria (Ahmad and Beg, 2011). Aqueous root extract (1 mg/ml) showed MIC ranging from 0.04 mg to 0.1 mg against Gram-positive American type culture collection (ATCC) strains of *S. aureus*, *Pseudomonas aeruginosa*, and *K. pneumonia* with a zone of inhibition values ranging from 14, 12, and 14 mm, respectively (Mahalingam and Kannabiran, 2009). When compared to the standard antibiotics ampicillin, tetracycline, and chloramphenicol (MIC values ranging from 9.5-21.6 mg/ml), *H. indicus* root extracts (petroleum ether, chloroform, methanol, ethanol, and water) showed moderate growth inhibition against 12 human pathogenic bacteria with MIC values ranging from 6.20-10.65 mg/ml (Sujatha and Anusha, 2010). The *H. indicus* ethanolic extracts were tested for antibacterial activity against *E. coli* using a colony-forming assay unit, as well as the effect of the extract on the bacterial membrane using fluorescence-activated cell sorting and a scanning electron microscope destruction of bacteria observed at extract concentrations of 400 and 500 g/ml (Saritha *et al*., 2015). Using standard disc diffusion methods, different extracts of *H. indicus* (petroleum ether, chloroform, methanol, ethanol, and aqueous) are tested for antibacterial activity against 15 human pathogenic microorganisms. When compared to standard antibiotics (ampicillin, tetracycline, and chloramphenicol, which have MICs ranging from 9.5 to 21.6 mg/ml), the extracts showed moderate inhibition with MICs ranging from 6.20 to 10.65 mg (Sujatha and Anusha, 2010). Various pharmacological proved that medicinal herbs could be effective natural antibacterial drugs against fish pathogens. Chitmanat *et al*. (2005) reported that Indian almond extract has the potential to inhibit the growth of tilapia ectoparasites and the bacterial pathogen *A. hydrophila*. Another medicinal plant *Rosmarinus officinalis* was found to be effective against *Streptococcus* infection in tilapia (Abutbul *et al*., 2004). The antimicrobial activity of five Chinese herbal extracts (*Stellaria aquatica*, *Impatiens biflora*, *Oenothera biennis*, *Artemisia vulgaris*, and *Lonicera japonica*) against thirteen bacterial fish pathogens, revealed that *Aeromonas salmonicida* and *Edwardsiella ictaluri* were the most sensitive to these extracts (Shangliang *et al*., 1990). The culinary herb cinnamon was found to have a large zone of inhibition against *A. hydrophila* causing infection in Nile tilapia (Ahmad *et al*., 2011). In addition, ethanolic extracts of *Piper guineese* (fruits) and *Xylopia aethiopica* (seeds) were active against the fungus *Candida albicans* and bacteria *B. subtilis*, *Escherichia coli*, *S. typhi* and *P. vulgaris* (Okeke *et al*., 2001). Citarasu (2000) performed a comparative study of methanolic extracts of three ayurvedic herbals viz. *Solanum trilobatum*, *Andrographis paniculata*, and *Psoralea corylifolia* which showed varying degrees of protection for *Penaeus* sp. against nine pathogens such as *Bacillus subtilis*, *Proteus vulgaris*, *Salmonella typhi*, *K. pneumoniae*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Vibrio* sp., *S. aureus* and *A. hydrophila*.

Conclusion

We investigated the antibacterial activity of different parts of *H. indicus*-- leaves, stems and roots. We have prepared various solvent extracts of three different parts of the *Hemidesmus indicus* such as hexane, ethyl acetate, ethanol and aqueous, evaluated the antimicrobial activity by standard agar well diffusion method, tested

against aquatic pathogens causing a major threat to the aquaculture industry, such as Gram-positive bacteria such as *Streptococcus agalactiae* and *Streptococcus iniae* and Gram-negative strains such as *Aeromonas hydrophila*, *Vibrio cholera* and *Pseudomonas aeruginosa* and two fungal strains such as *Aspergillus niger* and *Candida albicans*. The study concluded that the antimicrobial activity of plant parts varied depending on the solvents used, sample concentration, and microbes tested for the experiment. Overall, we can conclude that all parts contain antimicrobial compounds, with the root being the most potent antimicrobial drug. As a result, *Hemidesmus indicus* contains promising antimicrobial compounds that can be used in medicine and food applications in the field of aquaculture. However, more research is needed to document the bioactive compounds in the herb to improve its application.

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