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The impact of replacing antibiotics and improve the antimicrobial efficiency of aqueous extract *Hemidesmus indicus* against fish pathogen

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Abstract

A new area of biopharmaceutical research has been made possible by the plant investigation. In traditional medicine, medicinal plants have been used to treat a variety of ailments. Chemical structures that are uncommon in their terrestrial counterparts can be discovered in compounds obtained from herbal sources. Animals must utilise antibiotics carefully if we want to prevent the growth of microorganisms that are resistant to the antibiotics that are now being used. Additionally, fish growers must guarantee that fish are maintained in the optimal conditions for welfare and health. Furthermore, it is important to promote the development of new medications and the use of antibiotic substitutes. More natural products have reportedly been found to have antibacterial properties in recent years. To comprehend herbal remedies and their preparation, it is desirable to have knowledge of the chemical components of plants. The current study contends that the *Hemidesmus indicus* extract has important ramifications for the food and pharmaceutical industries as a source of raw materials for making dietary supplements and sophisticated chemical synthesis. As a result of this study's findings, it is possible to produce bioactive leads for the treatment of infections using plant extracts from the leaves of *Hemidesmus indicus*.

Keywords: plants, *Hemidesmus indicus,* bioactive, treatment, pharmaceutical

Introduction

Plants are abundant sources of several forms of medication because they produce a wide variety of bioactive chemicals. The majority of medications used in conventional medical systems today are either from natural sources or from semi-synthetic versions of natural materials. According to reports, medicinal plants are said to be rich in antioxidant molecules when they are growing in an ecologically unfriendly environment that includes factors like salinity, high temperatures, waterlogging, low oxygen, and sunshine. Phytochemicals such cinnamic acids, coumarins, diterpenes, flavonoids, lignans, monoterpenes, phenylpropanoids, tannins, and triterpenes are among these substances (Bandaranayake 2002; Thatoi *et al*. 2014) [12].

Studies on the phytochemistry of *H.indicus* Alkaloids, flavonoids, triterpenoids, and steroids have been found in leaf extract, and these secondary metabolites may also be to blame for the substance's bioactivity. Because they scavenge free radicals or activate antioxidant enzymes, several substances with antioxidant action help prevent cancer and mutation (Abeysinghe *et al*., 2006) [1].

Since antibiotics are the first line of defense against bacterial illnesses, they are crucial to modern medicine. Although the development of extended drug resistant (XDR) and multidrug resistant (MDR) bacteria is a major source of concern, antibiotic resistance is a long-standing phenomenon that predates any therapeutic use of antibiotics. The prolonged use of antibiotics in aquaculture increases the selective pressure on bacterial populations, even at antibiotic concentrations well below the minimum inhibitory concentration of the susceptible wild type population, and also increases HGT rates, including human and fish pathogens. Bacteria are becoming resistant to a wide variety of antibiotics as a result of natural processes and widespread anthropogenic activity. Because they are not biodegradable and are rather durable, antibiotics can remain in commercialized fish and shellfish for consumption. The present study aimed to utilize the plant which is one of the important sources of western ghates. They are widespread in tropical and subtropical regions (Chelliah, 2001; Tariq *et al.,* 2007) [3, 11].

Objectives of the Study

The goal of the current study was to make use of a plant that is a significant source in the Western Ghats. Plants have proven to be essential sources of many effective medicines throughout history, and they are crucial for the screening of new lead compounds. Traditional medicine uses medicinal plants extensively to cure severe ailments. *Hemidesmus indicus*, a medicinal plant, has also demonstrated antibacterial capabilities.

Materials and Methods

Sample collection

The plant *H indicus* was taken in the Western Ghats region, Kanyakumari district, Tamil Nadu. The plant samples (root) were properly cleaned in fresh water to eliminate soil and other undesirable elements before being air dried in the shade. The powdered dried plant roots were divided into smaller pieces, kept at 4 °C before analysis.

Preparation of Extracts

After drying, the *H indicus* sample was weighed and then chopped. Using a mixer grinder, the sample pieces were finely powdered. Five gram of the finely powdered samples were weighed and then dissolved in a variety of organic solvents, including 80% ethanol, methanol, and chloroform. It was combined consistently over the course of 48 hours at room temperature. Using Whatman No1 filter paper, the material that had been dissolved in each solvent was separated for use after 48 hours.

Phytochemical screening

Test for tannins

In a test tube, 10 ml of water and about 0.5 g of the extract were heated, and the mixture was then filtered. A few drops of 0.1% ferric chloride were added, and the coloration was checked for blue-black or brownish green.

Test for saponins

In a test tube, 0.5 g of the extract was mixed with 5 ml of distilled water. The mixture was aggressively agitated to check for a stable, long-lasting foam. Three drops of extra virgin olive oil were added to the mixture, which was vigorously shaken before being tested for the presence of an emulsion.

Test for steroids

An equal volume of concentrated sulphuric acid was introduced by the test tube's sides after one millilitre of the extracts had been dissolved in ten millilitres of chloroform. The sulphuric acid layer appeared yellow with green fluorescence while the upper layer turned red. This suggested that steroids were present.

Test for terpenoids (Salkowski test)

Two ml of chloroform were added to 0.5 g of the extract for each. To create a layer, concentrated H2S04 (3 ml) was carefully applied. The presence of terpenoids is indicated by the interface's reddish brown coloration.

Test for triterpenoids

Ten milligrammes of the extract were dissolved in one millilitre of chloroform, then two millilitres of concentrated H2SO4 were added. This mixture was then subjected to a triterpene test. Triterpenoids are present when reddish violet colour develops.

Test for anthraquinones

Sulphuric acid (H_2SO_4) was added to 0.5g of the extract to be cooked, and the mixture was immediately filtered. 5 cc of chloroform was added to the filter and shaken. One millilitre of diluted ammonia was added after the chloroform layer was pipette into another test tube. Color alterations in the final product were checked.

Test for flavonoids

Undiluted ammonia (5 ml) was added to a portion of the extract's aqueous filtrate. One millilitre of concentrated sulfuric acid was applied. When upright, a golden hue that vanishes suggests the presence of flavonoids.

Test for alkaloids

An extract of 0.5 g was boiled and filtered after being diluted to a volume of 10 ml with acid alcohol. I added 2 ml of diluted ammonia to 5 ml of the filtrate. To extract the base of the alkaloid, 5 ml of chloroform was added and gently shaken. 10 cc of acetic acid were used to remove the chloroform layer. Two pieces of this were separated. To one portion, Draggendorff's reagent was added, and to the other, Mayer's reagent. Alkaloids were thought to be present when a cream-colored precipitate or a reddish-brown precipitate formed using Mayer's or Draggendorff's reagent, respectively.

Test for cardiac glycosides (Keller-Killiani test)

A solution of ferric chloride in glacial acetic acid, 2 ml, was added to 0.5 g of extract that had been diluted to 5 ml in water. One millilitre of pure sulfuric acid was applied underneath this. A deoxysugar element typical of cardenolides was seen as a brown ring at the contact. Below the brown ring, a violet ring may occur, and just above the brown ring in the acetic acid layer, a greenish ring may form and eventually extend throughout this layer.

Fourier Transform Infra-Red (FTIR) spectroscopic investigation

The Fourier transform infra Red (FTIR) method was used to qualitatively examine the chosen hot water extract, such as *H. indicus*, for the active components. An infrared spectrometer that produces an intensity signal as a function of wavelength or spectral colour is called a Fourier transform spectrometer. The system is different from a traditional grating or prism spectrometer in that an interferogram is taken instead of immediately recording the spectral strength as a function of wavelength.

Antimicrobial activity of *H. indicus*

The Muller-Hinton agar diffusion method was used to assess the antibacterial activity of an extract of H indicus. We employed 24-hour-old broth bacterial cultures that were cultivated on nutrient agar plates. By using the pour plate approach, an aliquot (0.05 ml) of inoculums was added to the molten agar medium and poured into a Petri dish. After solidification, sterile cork-borers were used to make the necessary wells on agar plates and sterile gel punchers were used to make 6.0 mm diameter wells over the agar plates. 0.1ml of each extract was added serially to agar. For the purpose of observing antimicrobial activity, 24-48 hours of incubation at 28°C for fungal growth and 30°C for bacterial growth were maintained.

Results and Discussion

Phytochemical analysis

This study has identified the existence of phytochemicals thought to be active chemical components in medicines. Alkaloids, terpenoids, flavonoids, and anthraquinones, among other significant therapeutic phytochemicals, were found in the samples. According to the findings of the phytochemical investigation, the extract of *H. indicus* contained alkaloids, saponin, flavonoids, and Phenols. (Table1). The crude extract of *H. indicus* fluoresced clearly at various radiation levels, exhibiting what might be considered a typical fluorescence pattern. Standardization of plant material is necessary nowadays since plants and their chemicals have served as a useful source of inspiration for new therapeutic molecules. Table 1 summarises the findings of the preliminary qualitative phytochemical screening of *H. indicus*. Steroids, pholobatanins, amino acids, and aromatic acids were not detected in the water extract of *H. indicus*, but tannins, saponins, flavonoids, phenolic group, and carbohydrates [8].

Table 1: Phytochemical Analysis of selected plant leaf by standard protocol

S. No	Phytochemical Compounds	*H. indicus*
1.	Alkaloids	Positive
2.	Tannins	Positive
3.	Saponins	Positive
4.	Steroids	Positive
5.	Terpenoids	Negative
6.	Titerpenoids	Negative
7.	Flavonoids	Positive
8.	Anthraquinones	Negative
9.	Cardiac glycosides	Positive

Functional group analysis

Figure 1 shows the results of an investigation using Fourier Transform Infrared Spectroscopy for the active portions of the antibacterial extracts. The portion of *H. indicus* extracts that is active in hot water The I-R spectrum from produced the following peaks. The peaks represented the molecule's numerous functional groups.

One of the plant chemicals examined in *H. indicus* phenolic chemicals make up the majority of the root extract, which is also strong in tannin, alkaloids, saponin, and flavonoids. According to this study, functional group associations and fourteen effective peaks were found in the range of 4000 cm-1 and 450 cm-1. These peaks signify the presence of several organic functional groups, including phenols, alcohols, and alkyl groups. The therapeutic properties of pharmaceutical compounds may be due to the presence of these recognised functional groups.

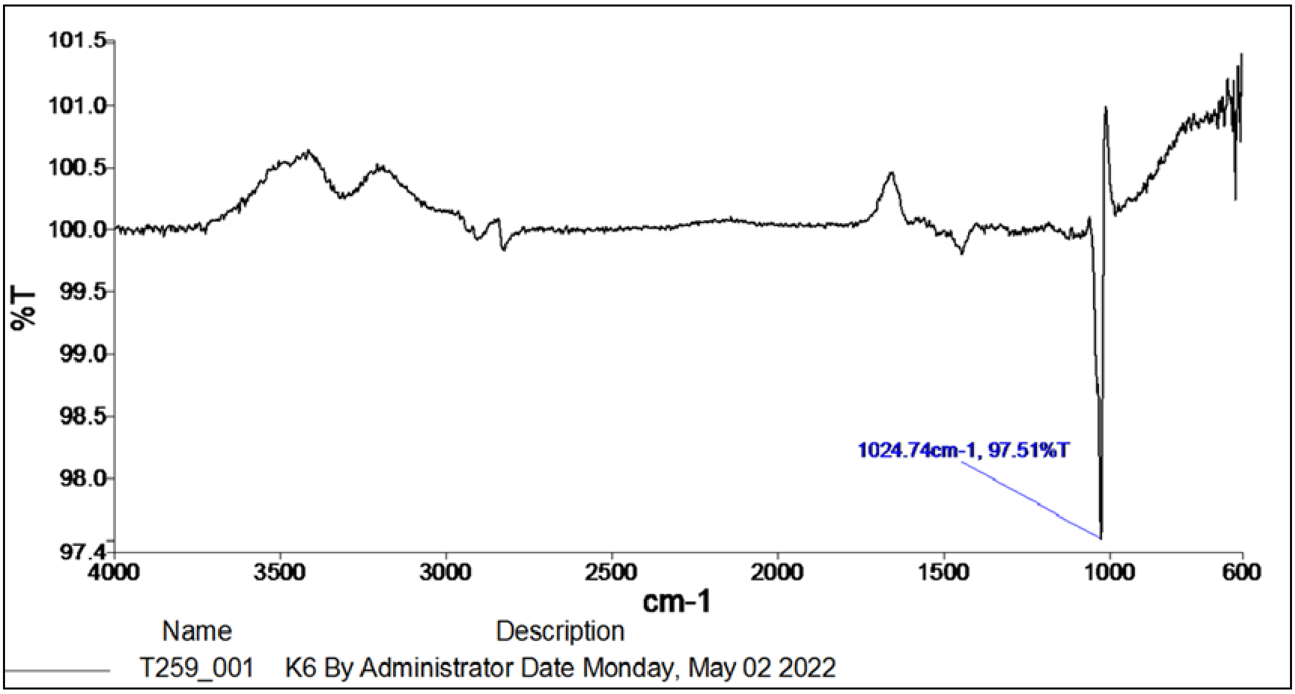

Fig 1: Active principles isolated from the extract of *H. indicus* through FTIR Spectroscopic analysis

Antibacterial activity of *H. indicus*

In Fig. 2, the antibacterial activities of the chosen extract (in hibitory zone of mm diameter) were shown. *H.indicus*. Amo ng the various extract concentrations (0 mg, 50 mg, and 100 mg), *A.hydrophila's* controlled the pathogen in zones of inhibition of 0.1, 3.4, and 6.1 mm, respectively.

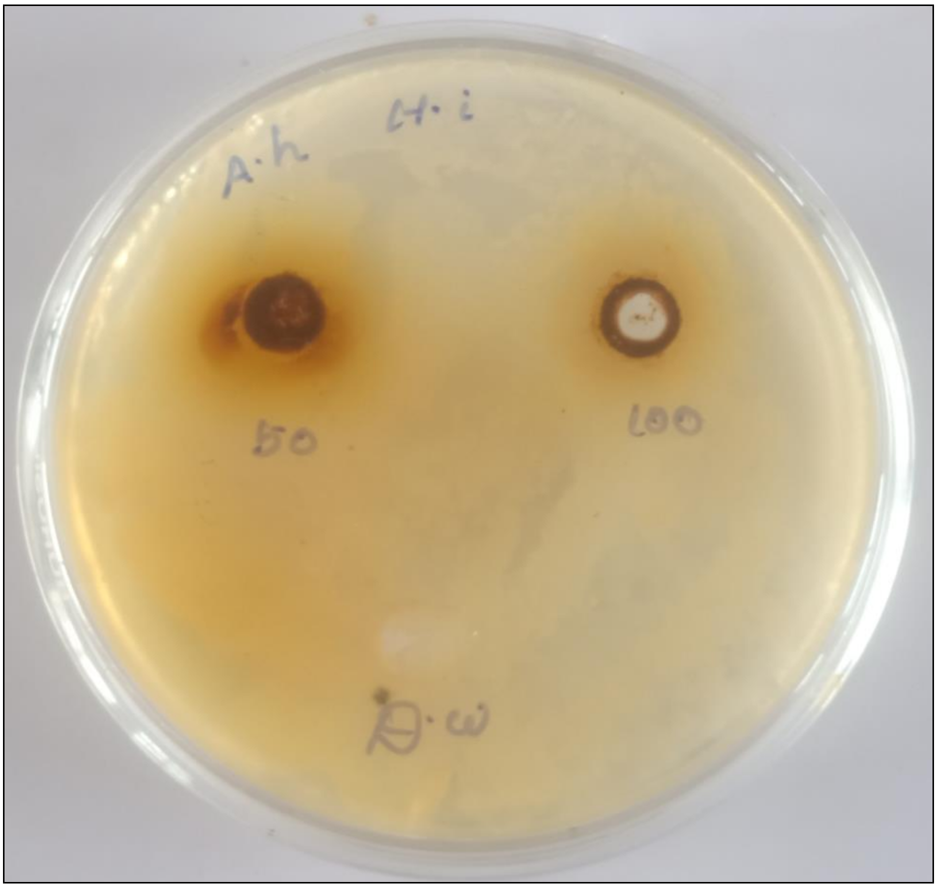

Fig 2: Antibacterial activity of *H. indicus* against *A.hydrophila*

Recent strategies for developing novel medicines from unexplored natural resources recommended plants as an important source of potentially useful chemicals (Harvey 2000) [6]. The presence of phenolics in *H. indicus* which are toxic to microbial pathogens especially shows the maximum activity against bacterial pathogens. Alkaloids present in this plant are used as basic drug agents for antispasmodic, analgesic and antibacterial effect (Okwu, 2004) [7]. Flavonoids found in this plants are preventing the oxidative cell damage and having strong anticancer activity (Okwu, 2004) [7]. Saponins present in these plants are considered to be antifungal agents and tannins prevent the growth of the microorganism by precipitating nutritional microbial proteins and also have the property of cholesterol binding and bitterness (Okwu, 2004) [7].

Conclusion and Recommendation

The findings showed that the *H indicus* under study included components with significant therapeutic value. As a result, it is possible to view the *H. indicus* plant's extracts as a reliable source for therapeutics. It is strongly advised that these plants be used in traditional medicine, and it is also proposed that more research be done to identify, purify, and isolate the medicinal ingredients that give these plants their effects. Additionally, more research is encouraged to clarify the potential mode of action. the *H.indicus* extract.

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