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ANTIMICROBIAL ACTIVITY OF ANALYSIS OF FRUIT EXTRACTS OF TERMINALIA BELLERICA

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Abstract

The medicinal plant Terminalia bellirica, which is highly regarded in traditional medical systems, has attracted a lot of interest due to the multitude of pharmacological qualities that it possesses. The purpose of this study was to explore the antibacterial activity of fruit extracts from Terminalia bellirica against a group of microorganisms that are clinically relevant. First, the extraction of bioactive substances was carried out using solvents of varied polarity, and then the results were evaluated with agar well diffusion and broth microdilution tests. According to the findings of our study, the fruit extracts of Terminalia bellirica exhibited considerable antibacterial activity against Gram-positive and Gram-negative bacteria. These bacteria included Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. In addition, substantial antifungal activity against Candida albicans was observed by the extracts. The effectiveness of the antimicrobial agent varied depending on the solvent that was employed for extraction, with methanol extracts displaying the highest level of effectiveness. An examination of the phytochemical composition revealed the presence of polyphenols, flavonoids, and tannins, all of which have the potential to contribute to the antibacterial activity that was detected. In addition to highlighting the potential of Terminalia bellirica fruit extracts as natural antimicrobial agents, this work offers useful insights into the antibacterial potential of these extracts and highlights the need of additional investigation into their use in drug discovery and development.

***keywords**: Antimicrobial, terminalia, bellerica*

Introduction

Since antimicrobial resistance has become an increasingly serious threat to the health of people all over the world, there has been an urgent demand for innovative therapeutic medicines. Over the course of the past several years, researchers have shifted their focus to the investigation of natural sources for antimicrobial chemicals as an alternative to the traditional antibiotics. Medicinal plants have emerged as a prospective reservoir of bioactive compounds with powerful antibacterial characteristics, and they are among the natural reservoirs that have been discovered. Terminalia bellirica, sometimes referred to as "Bahera" or "Bibhitaki," is a highly regarded medicinal plant that is a member of the family Combretaceae. It is native to the Indian subcontinent and has been utilised widely in traditional medical practices such as Ayurveda for a considerable amount of time. Particular attention has been drawn to the fruit of T. bellirica due to the wide therapeutic potential it possesses. This fruit is distinguished by its astringent flavour and pharmacological richness. The pharmacological activities of T. bellirica have been described in a great number of investigations. These activities include its antioxidant,

anti-inflammatory, and antibacterial characteristics. However, despite the increased interest, there have been very few systematic research into the antibacterial activity of T. bellirica fruit extracts. This is especially true with regard to the ability of these extracts to combat clinically relevant microbial infections. By conducting a comprehensive analysis of the antibacterial activity of fruit extracts obtained from Terminalia bellirica against a group of therapeutically important pathogens, the purpose of this work is to fill in this vacuum in knowledge. We intend to understand the range of antimicrobial activity demonstrated by T. bellirica fruit extracts by utilising a mix of extraction procedures and antimicrobial tests. This will allow us to build the framework for the possible development of these extracts as natural antimicrobial agents. The purpose of this introduction section is to present an overview of the antimicrobial crisis, the relevance of natural products in the process of drug development, and the reasoning for the investigation of Terminalia bellirica as a possible source of antimicrobial chemicals. In the following sections, we will look into the technique that was utilised, the results that were produced, and the ramifications of our findings in relation to the larger context of antimicrobial research and drug development.

MATERIALS AND METHODS

Hi-Media Pvt. Limited, located in Bombay, India, was the source of all of the chemicals and reagents that were utilised. Products made of glass were sourced from Borosil.

Collection of fruit

In the months of January and February 2013, the fruits of Terminalia bellerica were gathered from the locations in and around Coimbatore, which is located in the state of Tamil Nadu. An certified specimen of the fruit was stored in the Department of Botany at Avinashilingam University for Women in Coimbatore, Tamil Nadu, India. The fruit was also provided with a voucher specimen. In order to eliminate any dirt from the fruits, they were washed thoroughly under running tap water for two to three times. After that, they were allowed to dry in the shade at room temperature for a week. After the seeds were removed from the dry fruits, they were crushed into very small particles and placed in a container that was sealed. After that, they were kept at room temperature until they were used again.

Preparation of fruit extract

In order to homogenise the ground sample of Terminalia bellerica, ten grammes of the sample were weighed, and then 100 millilitres of petroleum ether, aqueous, and chloroform were each homogenised individually. After being shaken for a full 24 hours at room temperature, the crude preparation was centrifuged at a speed of 4000 revolutions per minute for a period of twenty minutes. Following this, the supernatant that contained the fruit extract was transferred to a beaker that had been pre-weighed, and the extract was concentrated by evaporating the solvent at a temperature of sixty degrees Celsius. After adding 10 grammes of the sample to 100 millilitres of distilled water, the mixture was shaken at a speed of 90 to 120 revolutions per minute for twenty-four hours at a temperature of thirty degrees Celsius. The combination was brought to a boil at a temperature of sixty degrees Celsius for three hours, and then it was concentrated to one-fourth of its initial volume. After that, the extracts were heated in a rotating evaporator while being subjected to a vacuum and lowered pressure in order to achieve a dryness. Following that, the crude extracts were diluted in a precise volume of dimethyl sulfoxide (DMSO) in order to achieve a final concentration of 20 milligrammes per five microliters. We kept the aliquot in storage until we needed to utilise it.

Culture media and inoculums preparation

During the process of cultivating the bacterial strains, the media that was utilised was Muller Hinton agar media or broth, which was purchased from Himedia in Mumbai, India. Following the inoculation of the Muller-Hinton broth with all of the bacterial cultures, the mixture was then incubated at 37 degrees Celsius for twenty-four hours. For the purpose of cultivating fungal strains, the media that was utilised was Rose Bengal Chloramphenical agar/broth provided by Himedia, located in Mumbai, India. All of the fungal cultures were placed in a loop that was filled with the Rose Bengal Chloramphenical broth, and the mixture was then incubated at room temperature for a period of seventy-two hours.

Antimicrobial assay

Well diffusion method

For the purpose of determining the antibacterial activity of the extracts, the agar well diffusion technique was utilised. In a nutshell, a sterile cork borer with a diameter of five millimetres was used to create five wells in Muller Hinton agar plates and Rose Bengal chloramphenical agar plates, respectively. A total of fifty microliters of bacterial and fungal inoculum were swabbed on the plates mentioned above using sterile swabs in independent batches. When using a micropipette, 20 microliters of each extract, a control solution (DMSO), and standard antibiotics (four milligrammes of chloramphenical for bacteria and four milligrammes of nystatin for fungi) were individually dispensed into the wells that were designated for them. After that, the plates were incubated at 37 degrees Celsius for twenty-four hours for the bacteria, and at room temperature (ten to thirty degrees Celsius) for five days for the fungal isolates. The samples were examined in triplicates, and the diameter of the zone of inhibition was measured in millimetres (mm). The results were represented as the mean plus or minus the standard deviation.

Phytochemical screening of the extracts

In accordance with the methodology described by, the extracts that were taken from the fruits of Terminalia bellerica were subjected to qualitative analysis in order to determine the presence of various phytochemicals. These phytochemicals included alkaloids, phenols, amino acids, flavonoids, saponins, tannins, quinones, carbohydrates, glycosides, steroids, and terpenoids.

FT – IR analysis

For the purpose of determining the different kinds of chemical linkages (functional groups), a technique known as Fourier transform infrared (FT-IR) is utilised. One of the characteristics of the chemical bond that may be observed in the spectrum that has been annotated is the wavelength of the light that is absorbed. The chemical bonds that are present in a molecule can be identified through the process of evaluating the infrared absorption spectrum. The dried powder of aqueous extract of Terminalia bellerica fruits, which weighed ten milligrammes, was placed in a mortar and pestle and crushed with two and a half milligrammes of dry potassium bromide (KBr) for the Infrared Fourier Transform (FT-IR) investigation. Using a micro-cup with an internal diameter of 2 millimetres, the powder that was collected was then placed into a Fourier transform infrared spectrometer that was adjusted at 26 degrees Celsius with a standard deviation of 1 degree Celsius. In order to analyse the materials, a Fourier Transform Infrared Spectrometer (Shimadzu, IR Affinity 1, Japan) was utilised to perform infrared scanning in the range of 4000–400 cm-1. A comparison was made between the spectral data that was collected and the reference chart in order to determine the functional groups that were present in the sample.

Microdilution method

The micro dilution approach was utilised in order to establish the minimum inhibitory concentration (MIC) by employing Terminalia bellerica extracts that were serially diluted in accordance with the NCCLS procedure [9]. Using 96-well plates, the aqueous extract was diluted to get a range of concentrations ranging from 100 mg/ml to 1.56 mg/ml. These concentrations were obtained in sterile Muller-Hinton broth. After adding the microbe suspension, which was 50µl in volume, to the broth dilutions, the mixture was then incubated at 37ºC for a period of 18 hours. The minimum inhibitory concentration (MIC) of each extract was determined by determining the lowest concentration at which there was no discernible growth of bacteria.

RESULTS

A summary of the findings collected can be seen in Table 1, which provides information on the growth inhibition that was caused by the fruit extract of Terminalia bellerica towards bacterial and fungal isolates. The findings of the experiments that were conducted for this study demonstrate that the aqueous extract was found to be more successful in controlling the development of bacterial and fungal organisms when compared to the chloroform and petroleum ether extracts, respectively. The zone of inhibition varied from 15 to 23 millimetres, and all of the bacterial and fungal isolates that were examined exhibited considerable activity against the aqueous extract and demonstrated significant activity. By measuring between 9 and 15 millimetres, the chloroform extract of the fruits of Terminalia bellerica demonstrated a moderate zone of inhibition against the bacterial and fungal isolates that were examined. In comparison to the microorganisms that were examined, the petroleum ether extracts of the fruits of Terminalia bellerica had a smaller zone of inhibition, measuring between 8 and 13 millimetres. When compared with the usual antibiotics that were tested (Chloramphenicol and nystatin), the extracts displayed a considerable zone of inhibition. On the other hand, the negative control (DMSO) did not reveal any zone of inhibition. It was discovered that the aqueous extract had the greatest zone of inhibition against Klebsiella pneumoniae (23 mm) and Aspergillus fumigatus (22 mm), whereas the petroleum ether extract had the least inhibition against Salmonella typhi (8 mm) and Aspergillus niger (9 mm). The microdilution approach was utilised in order to ascertain the minimum inhibitory concentration (MIC) of the extracts in addition to their ability to inhibit the bacteria. Because the aqueous extract had the greatest zone of inhibition, the minimum inhibitory concentration (MIC) was only evaluated using this extract. The minimum inhibitory concentration (MIC) values of the extract against selected bacterial and fungal isolates are presented in Table 2. When compared with other microbiological isolates, the aqueous extract was able to suppress the growth of E. coli and A. fumigatus at a concentration of 6.25 mg/ml, which was the least concentration required (Table 2). Table 2 displays the minimum inhibitory concentration (MIC) values for the conventional antibiotics against the microorganisms that were tested. The findings provide additional evidence that aqueous extracts are effective against all of the bacterial and fungal isolates that were placed under the microscope. This table contains a tabulation of the phytochemical analysis that was performed on the fruit extracts of Terminalia bellerica. It was discovered that flavonoids, tannins, alkaloids, and phenol were present in the substance.

Table 1: Antimicrobial activity of the fruit extracts of Terminalia bellerica

Microorganisms	Zone of inhibition diameter (mm) in				
	Petroleum ether	Chloroform	Aqueous	Positive control	Negative control

Escherichia coli	11.6±1.5	13.6±1.5	14.6±1.5	21.6±1.5	-
Pseudomonas aeruginosa	9.3±2.5	12.6±2.5	13.6±1.5	26±1.0	-
Klebsiella pneumoniae	12.6±1.5	14.3±2.0	22.6±2.5	18±2.5	-
Shigella flexneri	9.3±2.0	16	21.3±1.5	12±2.0	-
Salmonella typhi	8±2.0	8.6±1.5	10±1.5	10.6±1.5	-
Aspergillus niger	9±1.0	11.3±2	17.6±1.5	20±1.0	-
Mucor species	10.3±1.5	17	20.6±1.5	13.6±1.5	-
Aspergillus fumigatus	13.3±1.5	14.6±1.5	19.3±1.5	23.3±1.5	-
Rhizopus species	12±2.0	15±2.0	19.3±1.5	23.3±1.5	-
Aspergillus flavus	10.3±1.5	10.3±1.5	20.3±2.5	24±1.0	-

Positive control – Chloramphenicol (Bacteria), Nystatin (Fungi), Negative control – DMSO

Table 2: Terminalia bellerica aqueous extract and bacterial and fungal isolates were tested to determine the minimum inhibitory concentration of the extract.

Bacterial isolates	Concentration (mg/ml)	Standard Antibiotics (mg/ml)
Escherichia coli	6.25	12.5
Pseudomonas aeruginosa	25	25
Klebsiella pneumoniae	50	100
Shigella flexneri	12.5	50
Salmonella typhi	100	12.5
Aspergillus niger	50	25
Mucor species	25	50
Aspergillus fumigatus	6.25	12.5
Rhizopus species	50	12.5
Aspergillus flavus	12.5	100

Standard Antibiotics – Chloramphenicol (Bacteria), Nystatin (Fungi)

Table 3: Analyses of the fruit extract of Terminalia bellerica carried out using qualitative phytochemical analysis

Phytochemicals	*Terminalia bellerica* extracts		
	Petroleum ether	Chloroform	Aqueous
ALKALOIDS			
Dragendroff's Reagent	+	+	+
Hager's test	-	-	-
Wagner's Reagent	-	-	-
PHENOLS			
Ferric chloride test	+	+	+
Lead acetate test	+	+	+
AMINO ACID			
Ninhydrin test	-	-	-
FLAVONOIDS			
Schinoda's test	+	+	+
Lead acetate Test	+	+	+
SAPONINS			
Froth test	-	-	-
TANNINS			
Breamer's test	+	+	+
QUINONES			
Borntrager's test	-	-	-
CARBOHYDRATES			
Molish test	-	-	-
Fehling's test	-	-	-
GLYCOSIDES			
Legal's test	-	-	-
STERIODS/TERPENOIDS			
Libermann – Burchardt test	-	-	-

In the range of 400 to 4000 cm-1, the Fourier transform infrared spectra of the aqueous extract of the fruits of Terminalia bellerica indicated the presence of a great number of functional groups. The existence of –OH, –COOH, –NH, and C=O groups may be inferred from the fact that it displays a peak at 3950, 3749, 3417, 2924, 2854, 2376, 1720, 1627, 1442, 1381, 1226, and 1033 cm-1, respectively, as shown in Figure 1.

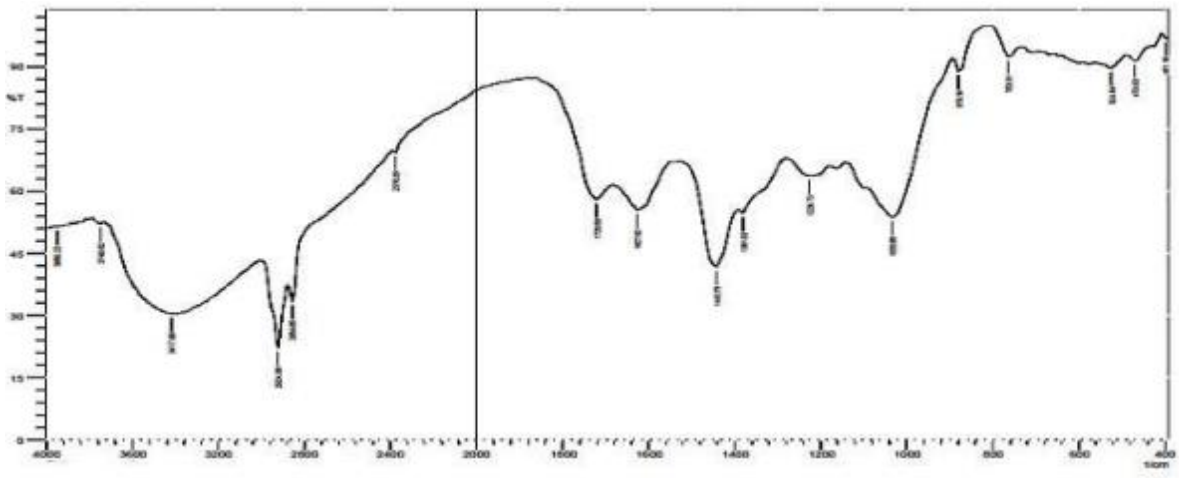

Fig. 1: FT –IR spectrum of the fruit extract of Terminalia bellerica

DISCUSSION

When it comes to public health concerns, infectious illnesses have emerged as the primary cause and a key source of worry. The formation of drug-resistant strains that are less susceptible to antibiotics as a result of mutation presents a challenge for researchers who are attempting to develop newer medications. In this particular context, the assessment of antibacterial compounds derived from a wide variety of medicinal plant sources is believed to play a crucial role. With the evidence of action against the bacteria under test, a scientific foundation has been established for the use of this plant in the treatment of a variety of illnesses in the local

community. The fact that the extracts were effective against the bacterial and fungal isolates that were examined may suggest that they have a wide range of activity capability. Because of the prospect of discovering therapeutic compounds that will be effective against organisms that are resistant to many drugs, this observation is of great significance. A traditional application of the fruit extract of Terminalia bellerica is supported by the findings of the study, which also reveals the existence of chemicals with antimicrobial characteristics that have the potential to be utilised as antimicrobial agents in innovative medications for the treatment of microbial disorders. This antimicrobial activity on bacterial and fungal isolates was validated by the aqueous extract of the fruits of Terminalia bellerica. This finding suggests that the phytochemicals included in the extract may be able to deactivate a variety of cellular enzymes that play an important part in the metabolic pathways of these pathogens. It has also been shown that phytochemicals have the potential to denature the proteins that are located in the cells, which therefore disrupts the normal processes that occur inside the cells. Phytochemicals are a class of non-nutritive compounds that are found in plant extracts. These phytochemicals exhibit biological activity and have the potential to have a therapeutic index that is of great value. It has been shown that certain phytochemicals exhibit a wide variety of actions, which may be beneficial in the prevention of chronic illnesses. The fruit extract of Terminalia bellerica was subjected to phytochemical analysis, which revealed the presence of flavonoids, alkaloids, tannins, and phenols. Possibly, the phytochemical alkaloid that was present in the fruit extract was responsible for inhibiting the microbe. This was accomplished by preventing the enzymes that were responsible for the creation of energy, disrupting the integrity of the cell membrane, and preventing the synthesis of structural components. There is a possibility that the presence of phenol, which may have caused swelling, plasma leaking and leakage, distortion, aberrant branching or fusing, and wrinkles in the hyphae, was responsible for inhibiting the growth of the fungus. It is possible that the presence of tannins in the fruit extract of Terminalia bellerica hindered the growth of germs by precipitating the microbial protein and rendering nutritious proteins inaccessible for the bacteria. Tannins have also been discovered to form irreversible complexes with proteins that are rich in proline, which results in the suppression of cell protein production. This information has been reported. When it comes to the medicinal characteristics of Terminalia bellerica, which have a high therapeutic content, it is possible that the presence of distinctive functional groups is responsible for these properties. It is important to note that the establishment of the respective antibacterial potential and the toxicological assessment of these extracts are being carried out with the intention of developing innovative chemotherapeutic drugs that will be utilised in the future.

CONCLUSION

According to the findings of the research, the traditional usage of fruit extracts is supported by the fact that these extracts include chemicals that have antibacterial characteristics and can be utilised in the development of innovative medications for the treatment of microbial infections. Further pharmacological assessments, toxicological investigations, and the possibility of isolating the therapeutic antibiotic from this fruit are the difficulties that lie ahead in the future.

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