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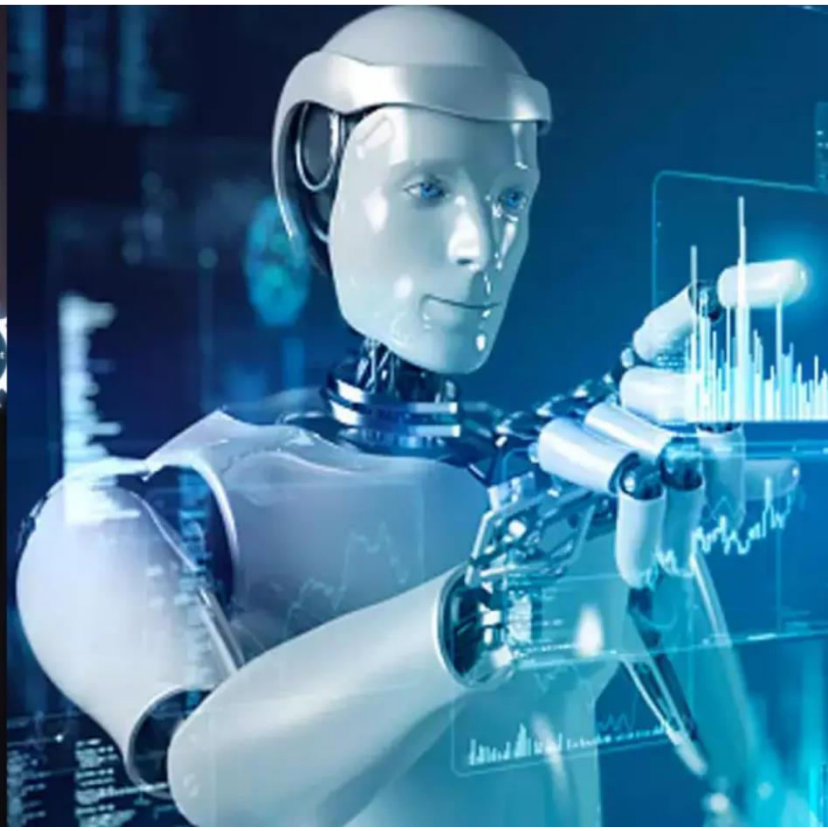
ISSN(O): 2319-8753
ISSN(P): 2347-6710



International Journal of Innovative Research in Science Engineering and Technology (IJIRSET)

(A Monthly, Peer Reviewed, Refereed, Scholarly Indexed, Open Access Journal)





Impact Factor: 9.935

Volume 15, Issue 10, October 2026

International Journal of Innovative Research of Science, Engineering and Technology (IJIRSET)

Impact Factor 9.935

|www.ijirset.com |A Monthly, Peer Reviewed & Refereed Journal| e-ISSN: 2319-8753| p-ISSN: 2347-6710|

Volume 15, Issue 10, October 2026

|DOI: 10.15680/IJIRSET.2026.1510104|

In vitro and *in vivo* Evaluation of Antibacterial Activity of *Phyllanthus niruri* LEAVES

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ABSTRACT: Hydro-methanolic extracts of *Phyllanthus niruri* leaves were tested for antibacterial action against a clinical isolate of E. coli. The excerpts shown considerable antibacterial action in vitro at concentrations of 25 mg, 50 mg, 100 mg, and 200 mg per ml, as well as *in vivo* at doses of 100 mg and 200 mg per kg. In vitro, the 200 mg per ml leaf excerpt prevented the most bacteria, followed via the 100 mg/kg *Escherichia coli*. *In vitro,* the greatest ZOI was seen at 200 mg per ml. The minimum inhibitory concentration (MIC) of the plant excerpts for *Escherichia coli* decreased as the concentration decreased, with the leaf extract containing 25 mg/ml against E. coli. Similarly, the minimum bactericidal concentration (MBC) demonstrated decreasing bactericide effects with decreasing concentration, with leaf having 25 mg/ml for *Escherichia coli.* The in vivo study revealed that the leaf extract had antibacterial activity against *E. coli* at dosages of 200mg/kg and 100mg/kg; the leaf excerpt had more action. In vivo, the 100 mg/kg dose reduced Escherichia coli colonies the most. A preliminary phytochemical showing of *Phyllanthus niruri* leaves revealed the occurrence of flavonoids, tannins, saponins, steroids, and triterpenoids. The presence of flavonoids, tannins, saponins, steroids, and triterpenoids in the plant's leaf excerpts indicates its medicinal value.

KEYWORDS: ***Phyllanthus niruri***, Antibacterial, *Escherichia coli, Nitrofurantoin*.

I. INTRODUCTION OF MICROORGANISM:

The Turkish Language Association Dictionary of Veterinary Medicine Terms defines as the microbes is alive entities such as microscopic algae , fungi, protozoa, and bacteria. While there has remained evidence about some of the effects and applications of microbes for centuries, microbes were firstly observed and considered in 1675, with Leewenhoek's creation of the optical microscope. In his investigations on the fermentation stage, Louis Pasteur demonstrated various roles of microbes in the creation of bad flavors in winemaking over the course of around 200 years, concluding that hotness therapy has an result on the death of surplus microbes. Pasteurization is a practice that is still widely recognized nowadays. At the time, Robert Koch's work also produced important influences to microbiology, and his work in these field has continual with quick pace till today.[1]. Microbes are classified into biological groupings such as fungi (molds and yeasts),viruses, bacteria, microscopic algae, and protozoa.

1.1. Protozoa

They are microscopic organisms with a single cell that can be found on land or in water. They exhibit animal traits like as mobility, food consumption, and lack of a cell wall. Certain protozoa obtain their nutrition in liquefied form via immersion.

1. 2. Microscopic Algae:

Maximum algae are classified as plant-like protists since they include chloroplasts and obligate cellulose cell walls. Over 7,000 types of green algae have been designated. The majority of them are water, existing in both clean water and seawater.

1. 3. Viruses

Because viruses differ from other microbes in their structure and general properties, they do not fit into any of the categories listed above.

1. 4. Fungi

Fungi are characterized by their filamentous eukaryotic cell structure. They have cell walls similar to plants, and the majority of them never move. Because they less photosynthetic colors, they receive nutrition since organic sources commonly via immersion. [2].

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1. 5. Bacteria

Bacteria is a microbes that can be originate in virtually any habitat. Certain of them spread ailments like typhoid fever, TB, and leprosy etc. In adding to these hazardous behaviors, they likewise provide numerous benefits. For sample, they convert organic deceased wastes into useable resources. This avoids the harm of important nutrients. The most basic unique qualities are their shape and dimension. Dimension differs from 1 to 10 µ. Maximum bacteria are prepared feeders. They analyze nearby organic nourishment bases through their enzymes, transform them into humble organic chemicals, and then engage them.[3]

1.5.1. Classification of Bacteria Based on Shape

1.5.1.1. *Coccus Bacteria*:

***Spherical bacterium*:**

These bacteria can be motile or non-motile.

Coccus bacteria can take various forms, including the ones listed below.

Monococcus*:*

∟ The bacteria may exist as single cell. e.g. *Micrococcus flavus*

Diplococcus:

∟ The bacteria present in the form of pairs, e.g. *Diplococcus pneumonia.*

Tetracoccus:

∟ Four coccus bacteria are grouped together, e.g. *Gaffkya tetragena*.

Streptococcus:

∟ Coccus bacteria present in the form of chain, e.g. *Streptococcus pyogenes.*

Staphylococcus:

∟ Coccus bacteria present in the group form, e.g *Staphylococcus aureus*

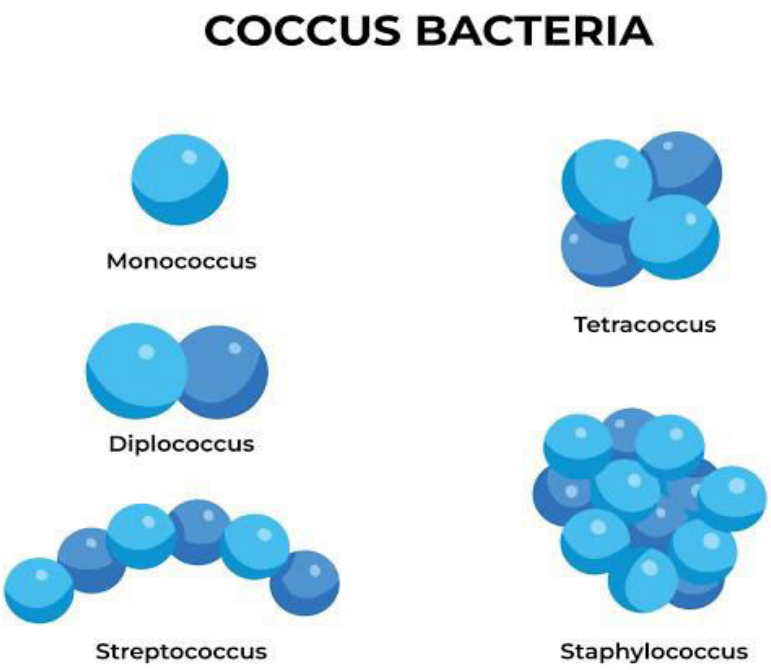


Figure No. 1. ***Coccus bacteria***

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1.5.1.2. Bacillus Bacteria:
These bacteria are rod-shaped.
These bacteria can be motile or non-motile.
Bacillus bacteria can take the following forms:
Monobacillus: A bacillus that exists as a single cell.
Bacilli that form pairs are known as diplobacillus.
Tetrabacillus bacteria form groups of four,
While *streptobacillus* bacteria form chains.

1.5.1.3. Spirillum Bacteria:
These microorganisms are spring-like.
These bacteria always exist singly. For example, *Spirillum volutans.*

1.5.1.4. Vibrio:
These bacteria are a little bent and resemble a 'comma'. As a result, they are also known as "comma bacteria. As a result, they are also known as "comma bacteria." These bacteria have a flagellum at their tip. For example, *Vibrio cholera* [4].

1.5.2. Classification of Bacteria Based on Occurrence or Absenteeism of Flagella
These are divided into the following types based on whether they have flagella or not:

1.5.2.1.*Atrichous*:
Corynebacterium diptherae is an example of a bacterium without flagella.

1.5.2.2) *Monotrichous*:
These bacteria have one flagellum linked to one end of the bacterial cell, such as *Vibrio cholera*.
.
1.5.2.3) *Lophotrichous:*
These bacteria, such as Pseudomonas, have a cluster of flagella attached to one end of the bacterial cell.

1.5.2.4) *Amphitrichous*:
In these bacteria, a cluster of flagella emerges since together ends of bacterial cell, such as *Rhodospirillum rubrum*.

1.5.2.5) Peritrichous:
Flagella are uniformly dispersed throughout the bacterial cell, as shown in *Bacillus.*

1.5.3. Classification of Bacteria on the Basis of Gram Staining
Hans Christian Gram, a Danish bacteriologist, devised a bacterial staining procedure in 1884.
This classification of bacteria is based on the capability of bacterial cell walls to retain crystal violet pigment.
Bacterial samples are stained with crystal violet, then iodine, and finally with alcohol.
The slides are then counter-stained with safranin.
The bacteria show distinct staining properties and are classed as follows.

1.5.3.1. Gram Positive Bacteria:
The bacteria must a substantially heavier peptidoglycan-based cell wall.
As a result, alcohol does not elute iodine or crystal violet from the thick cell wall.
As a result, when stained with the counterstain saffranine, these germs look purple.

1.5.3.2. Gram Negative Bacteria:
These are thinner cell wall of peptidoglycan than Gram-positive bacteria.
As a outcome, crystal violet is simply eliminated from the bacterial cell wall.
When stained with the counterstain saffranine, these bacteria look pink or red. Bacteria can be recognized by their shape and staining [5].

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1.5.4. *E.coli*:

E.coli is a gram-ve straightforward rod (1.1-1.5µm×2.0-6.0 µm) that can exist alone or in pairs. In this work, conducted in Microbiology Unit of DNS Hospitals Pvt. Ltd., Indore, we attempted to isolate *E.coli*, a gram-ve bacillus, from several humanoid medical cases. The *E.coli* species was termed after *Escherichia*, who was the firstly to characterize the colon bacillus with this term in 1885. *E. coli* is supplementary classified into biotypes and serotypes. *E.coli* is gram-ve, straightforward, rod-shaped, organized one by one or in couples, and motile with peritrichate flagella. That are develops at 37 degrees Celsius on culture mediums such as, Blood Agar MacConkey Agar, and Nutrient Agar.

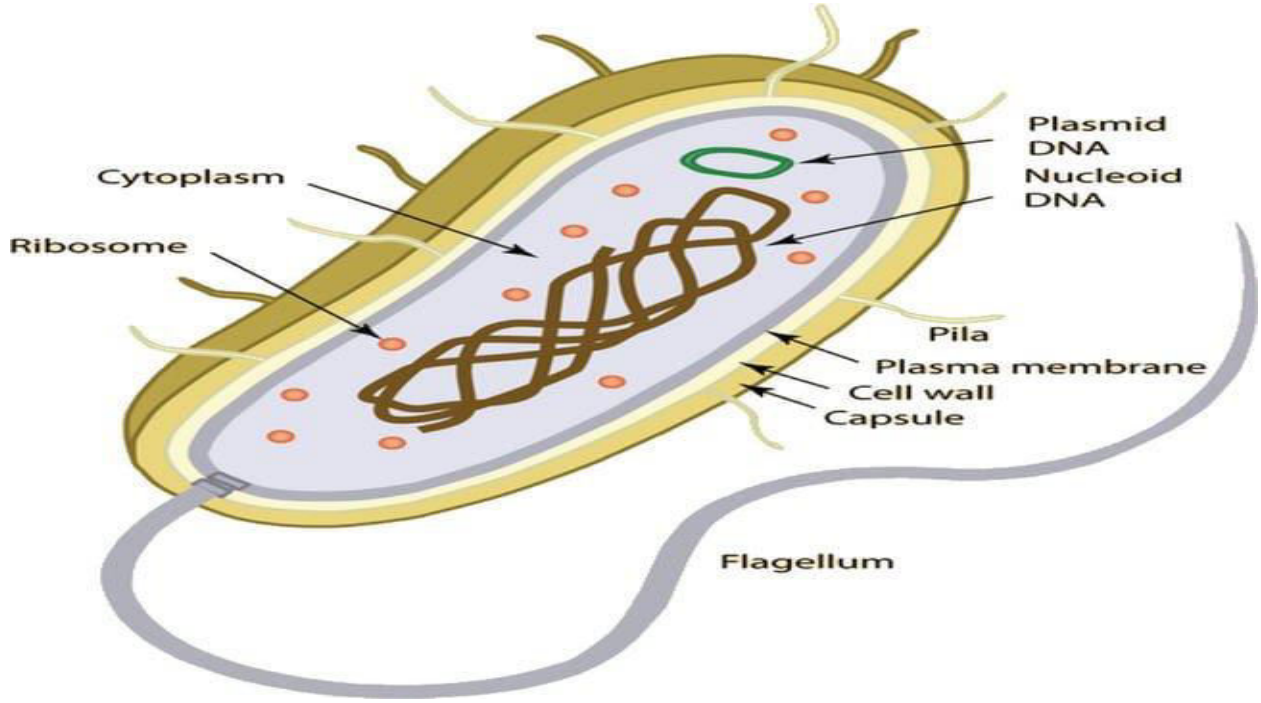


Figure No. 2. : *E. coli.* Bacteria

1.5.4.1. Taxonomic classification of bacteria

Table No. 1. : Taxonomic classification of bacteria

Class	*Gamma proteobacteria*
Genus	*Escherichia*
Kingdom	*Bacteria*
Phylum	*Proteobacteria*
Domain	*Bacteria*
Phylum	*Proteobacteria*
Order	*Enterobacteriales [6]*

1.5.4.2.*Enteropathogenic EPEC*: These are firstly disturbs newborns and younger kids in resource-limited situations, resulting in irregular and widespread outbursts. These are first pathogen recognized as the cause of water-logged diarrhea.

1.5.4.3. *Entrotoxigenic ETEC*: These are frequently detected in foodstuff and H2O in locations lacking proper sanitation, and it causes waterlogged diarrhea in resource-limited situations.

1.5.4.4. *Enteroinvasive EIEC*: These are rare causes diarrhea due to huge inoculums requisite, while it may be underdiagnosed.

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1.5.4.5. *Enterohemorragic EHEC*: Massive diarrhea outbreaks are caused when contaminated foods, such as spinach, shoots, lettuce, fruitlets, uncooked dairy goods, and raw grouse, are consumed.

1.5.4.6. *Enteroaggresive EAEC*: Acute and chronic watery diarrhea are caused by this organism, which is increasingly being recognized as a causative agent of traveler's diarrhea

II. METHODOLOGY

2.1. Select and collection of plant materials:-
The plant was designated for the proposed education based on its old-style medicinal uses, and the plant material was collected from the herbal garden of R. K. Pharmacy College, Sathiaon, Azamgarh, Uttar Pradesh

2.2. Authentication of plant:-
Department of Botany institute of science, Banaras Hindu University, varanasi India, permitted the plant. For future reference, a (voucher specimen no. *Phyllantha.* 2026/01 was set at the herbarium of India).

2.3. Requirements:-

Table no. 2. List of chemicals, instruments, drugs and bacteria:

Instruments/Glassware	Chemicals	Drugs	Bacteria
Antibiotics zone reader Scale	Beef extract Peptone Nacl Agar Distilled water Lactose Bile salt Neutral red Crystal violet	Nitrofurantoin	*E. coli*
Petri dish	Keller-killiani test		
Condenser			
Desiccator	Benedict test		
Grinder	Molisch's test reagents		
Mortar and pestle	Methanol Reagents and Distilled Water		
Digital pH meter	Biuret		
Soxhlet extractor	Ninhydrin		
Heating mantle	Foam test		
Oral cannula	Ferric chloride test		
Syringe Needle	Mayer's, Wagner's,		
Beaker	Shinoda		
Mesuring cylinder	Hager's		
Glass rod Spatula Pipette Weighing Balance Digital Weighing Balance	Lead acetate		

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2.4. Specimens collection:
Average samples were collected from 25 patients, from R. K. Ayurvedic Medical College Sathiaon Aamgarh. Swab specimens were aseptically transferred under make cold situations to the test for examination.

2.5. Isolation of E. *coli*:
Rendering to the method described by MacFadden , swabs were sensibly collected from the infected sites and engaged in tubes having transport medium to preserve moisture during transportation to the laboratory. Every specimen was subsequently inoculated onto MacConkey agar for the isolation of Escherichia coli. The inoculated plates were then incubated aerobically at 37°C for 24 hours.

2.6. Identification of *E. coli*:
2.6.1. Cultural Investigation: The colonial morphology of the isolates was examined on MacConkey agar. Colony colour, size, and edge characteristics were documented subsequent incubation at 37°C for 24 hrs.

2.6.2. Microscopic Examination: Rendering to the technique defined via Atlas et al., a single colony from every isolate was transferred onto a fresh glass slide, fixed, and subjected to Gram stain. The stained preparations were subsequently inspected underneath a light optical microscope to assess the morphological and staining characteristics of the bacterial isolates.

2.7. Biochemical tests
2.7.1. Catalase test
A singular colony from each bacterial separate was transferred onto a clean glass slide and mixed with 1.0 mL of 3% H2O2 solution. The formation of gas bubbles was considered indicative of a positive catalase reaction.

2.7.2. Oxidase test
The test was performed utilizing sieve paper moisturized with a little drops of newly make tetramethyl-p-phenylenediamine dihydrochloride solution. A clump of cells was aseptically collected from the slant culture using a sterile wooden stick and applied to the moisturized paper. The improvement of a violet or purple color in ten seconds was considered indicative of a positive result.

2.7.3. Indole check
A colony was inoculated into peptone water broth and incubation in a shaker incubator at 37°C for 24 hrs. Following incubation, a little drops of Kovac's reagent were added to the broth. The development of a pink-coloured ring was measured indicative of a positive result.

2.7.4. Methyl-red test
A bacterial colony was inoculated into MR-VP broth and incubated at 37°C for 24 hours. Afterward incubation, 3–4 droplets of methyl red chemical were added to the broth. The development of a red colour in the medium was considered indicative of a positive result.

2.7.5. Vogas-Proskauer test
A bacterial colony was inoculated into MR-VP broth and incubated at 37°C for 24 hrs. Following incubation, 2 droplets of VP1 reagent and 4 drops of VP2 reagent were added to the broth. The development of a red colour within 15 minutes was considered indicative of a positive result.

2.8. Preparation of extracts:-
Coarsely powdered plant leaves (500 g) were extracted exhaustively with a hydro-methanolic solvent using a Soxhlet apparatus at 60°C for 48 hours. The resulting extract was filtered through Whatman No. 1 filter paper. Complete solvent removal was achieved under reduced pressure using a rotary vacuum evaporator, yielding a powdery extract. The concentrated extract was subsequently stored in a vacuum desiccator for further use. [7]. HME was applied for antibacterial activities via various dosage phases of 25, 50, 100 & 200 mg per kg orally *In- vivo* study. These dosages are also applied for *In-vitro* study (cup plate method). The outcomes were compared by standard antibacterial agent Nitrofurantoin (5 mg/kg oral).

All test agents were suspended in water, which was used as the vehicle.

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2.9. Calculating of % Extractive Yield:
The **%** extractive yield will be designed the sum of excerpt obtained since the coarse powdered leaves material used for extraction. The final excerpt will be considered using an weighing balance. The initial weight of the plant coarse powder and the finishing weight of the excerpt will be recognized [8]. The **%** yield will be calculated through using the following formula:
Extractive yield (%) = Weight of extract obtained /Weight of powdered stem bark used ×100

2.10. Pre-formulation screening of extract:
2.10.1. Percentage yield of extract: The hydro-methanolic excerpt yield was measured as a percentage.

2.10.2. Physical characterization of excerpt: *Phyllanthus Niruri* **Leaves** excerpt was tested for its appearance, including colour, texture, and aroma

2.10.3. Determination of pH of extract: - The pH of the Phyllanthus niruri leaf excerpt was determined utilizing a calibrated digital pH meter. Entirely measurements were completed in three time.

2.10.4. Phytochemical analyses of excerpt: The phytochemical constituents of the hydro-methanolic excerpt of *Phyllanthus niruri* leaves were identified through qualitative analysis. A preliminary phytochemical screening was performed according to established procedures

2.11. Methods for phytochemical screening tests:-
The HME were primary phytochemical showing for detection of present of flavonoids, tannins, saponins, steroid and triterpenoid.

2.11.1. Test of alkaloids:
Mayer's test: On a steam shower, 0.4 g HME was mixed by 4 ml 1 percent HCL, and the mixture was filtered. A couple of droplets of Mayer's substance were add to few ml of filtrate. White of creamy white precipitous indications the occurrence of alkaloid.
Wagner's test: On a steam bath, 0.5 g HME was mixed with five ml 1 percent HCL and solution was filtered. A couple of droplets of Wagner's substance were added to one ml of filtrate. Brown or deep brown colored showed occurrence of alkaloids.
Hager's test: On a steam bath, 400 mg HME was mixed by using 5 ml 1 percent HCL and filtered. A few drop of Hager's reagent were added in 1 ml of excess. Occurrence of alkaloids showed Yellow crystalline ppt. [9].

2.11.2. Test of flavonoids:-
Shinoda test : Take small amount of Plant extract & liquefied in 5mL 95 % alcohol , add two droplets concentrated HCl and 0.4 g magnesium turnings. Improvement pink pigment detected in the presence of flavonoids.
Specific test: A test tube, taken was sample 0.5 ml. A lesser portion of magnesium or zinc ribbon was added. From that point onward 5-10 droplets concentrated HCL was added. The solution was heated for some minutes. Flavones, flavanols, and flavanones are shown through the Improvement of orange to red, red to ruby, crimson to magenta indicates.
Lead acetate test: A little quantity plant excerpt and add lead acetate solution was engaged in a test tube, develop yellow colored precipitous is made in the presence of flavonoids .

2.11.3. Test of Proteins and Amino acids:-
Biuret test: In test tube two ml plant excerpt + one droplet of two percent copper sulphate sol. + one mL of 95% ethanol + KOH bits was engaged. When proteins and amino acids presence a pink colored improvement.
Millon's test : Dual mL filtrate + two droplets of Millon's substance was mixed in test tube. In the attendance of proteins and amino acids a white precipitous improve.
Ninhydrin test: In a test tube, 2ml filtrate was mix using two droplets ninhydrin solution. When proteins and amino acid present, purple colour develop.

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2.11.4. Test of glycosides:-

In a general test:

A smaller quantity of the excerpt was liquefied in 1 ml of H2O and 1–2 droplets of an aqueous NaOH solution. Yellow pigment makes glycosides visible.

Legal's test for cardiac glycosides:-

In dual ml of sodium nitro-pruside solution (0.5 percent),100 mg of the extract was liquefied. Basic preparation of the mixture involved NaOH (0.2N) solution. Heat, glycosides are present and create pink to crimson colour.

Test of Baljet:

A small amount of the Baljet reagent was introduced along with the plant material. Due to the presence of cardiac glycosides, a yellow-orange tint developed .

2.11.5. Test of triterpenoids:-

Salkowski test: In a test tube, extract and couple droplets of concerted H_2SO_4 (stirrer well and permitted to stand). Improve Golden yellow film in the existence of triterpenoids .

2.11.6. Test of tannins:-

Gelatin test : Plant remove liquefied with five ml distilled water + one percent gelatin solution + 10% sodium chloride was added. Improve white precipitous existence of tannins.

2.11.7. Test of steroid:-

Libermann- Burchard's test : 2 ml plant extract and dissolved in 1ml of chloroform in test tube. Two ml of acetic anhydride and one ml of concerted H_2SO_4 was added. Existence of steroid develop light green color [10].

2.11.7. Test of saponins:-

Foam Test: Small amount of plant extract was shaken in a test tube with a little amount of water , the foam produced persisted for 10 minute. The presence saponins

2.12. *In Vitro* Assay:

2.12.1. Antibacterial Activity Test:

The antibacterial susceptibility of the plant extract was evaluated by the agar well diffusion technique [95]. Before testing, the bacterial isolates were cultured in nutrient broth for 18 hours and standardized to a concentration of \(10^6\) CFU/mL for Escherichia coli. Mueller–Hinton agar was ready rendering to the producer's instructions by liquefying 38 g of the medium in 1,000 mL of distilled water. The mixture was heated gradually until complete dissolution was achieved. Aliquots of 20 mL were dispensed into hygienic bottles and sterilized by autoclaving at 121°C for 15 minutes. The sterilized medium was then allowable to cool to room temperature. A volume of 0.1 mL of every test bacterial suspension involving \(10^6\) CFU/mL was added to bottles comprising 20 mL of molten Mueller–Hinton agar. The contents were mixed carefully and poured into labeled, sterile Petri dishes, where they were allowed to solidify. The agar plates were surface-dried at 35°C for 15 minutes. Subsequently, wells measuring 6 mm in diameter were created in the inoculated agar by a sterile cork borer. Plant extract concentrations of 200, 100, 50, and 25 mg/mL were prepared by serial dilution. Using a micropipette, 1,000 µL of each extract concentration and the standard antibiotic nitrofurantoin were introduced into the designated wells. Nitrofurantoin at a concentration of 50 µg/mL was used as the reference standard. The plates were allowed to stand for 60 minutes to facilitate the preliminary diffusion of the extracts into the agar [96]. Incubation was subsequently carried out for 18–24 hours. Finally, the antibacterial activity was assessed by measuring the diameters of the inhibition zones in millimeters (mm).

2.12.2. Minimum Inhibitory Concentration (MIC):

The minimum inhibitory concentration (MIC) was determined rendering to the procedure described by Ndukwe et al. (2007) [97]. Nutrient agar was ready following the industrialist's instructions at a standard concentration of 38 g per 1,000 mL of distilled water. Double-strength medium was ready via liquefying 38 g of the agar in 500 mL of distilled water, monitored via gradual warming till complete dissolution was achieved. Successively, 10 mL portions of the prepared medium were transferred into sterile bottles and disinfected by autoclaving at 121°C for 15 minutes. The decontaminated medium was then allowable to cool to room temperature.

The graded excerpt solutions were thoroughly mixed with the molten double-strength nutrient agar. The resulting mixtures were transferred into appropriately labeled, sterile Petri dishes and allowable to harden for one hour. Extract

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amounts of 200, 100, 50, 25, 12.5, 6.25, and 3.125 mg/mL were ready through serial dilution. The agar plates were surface-dried at 35°C for five minutes and then inoculated with the test isolates. Incubation was carried out at 37°C for 18–24 hours. Subsequent incubation, the plates were tested for the attendance or absence of microbial growth. The MIC was definite as the lowermost excerpt dose at which visible development of the test organism was prevented.

2.13. *In Vivo* Assay:

7.13.1. Animal:

Both gender mice aged 6–8 weeks and balancing 15–25 g were obtained from the random-bred colony at Banaras Hindu University (BHU) and utilized for the study. The mice were housed in the animal facility of R. K. Pharmacy College, Sathiaon, Azamgarh, in a temperature-controlled room kept under a 12-hour light/12-hour dark cycle. Food and water were providing ad libitum, and the mice were allowable to adjust to the research laboratory conditions for more than one week beforehand the trials were conducted.

2.13.2. Evaluation of Antibacterial Action:

The antibacterial action was determined *in vivo* utilizing the methods designated via Hosseinzadeh et al., 2007 [98], with minor changes. 12 mice, male and female, aged 6 to 8 weeks (15-25g), were shared into four sets of 3 mice every one. Three mice in every group were infested intraperitoneally with 0.1ml (106cfu/ml) of Escherichia coli suspension and housed in diverse cages. The 24 hrs. later, a specimen (blood) was collected from the mice tail and cultured on a specialized medium for Escherichia coli (MacConkey agar). Escherichia coli colonies grew, showing that the test organisms had already infected the animals. The 24 hrs. later, every group got separate treatments. The groups were considered as follows:

Group 1 (0.4ml of Nacl against *E. coli.)*

Group 2 (5mg/kg nitrofurantoin against *E. coli)*

Group 3 (200mg/kg leaves Excerpts against *E. coli.)*

Group 4 (100mg/kg leaves Excerpt against *E. coli.)*

After 72 hours, aspirated specimens from the mouse tail were collected and cultured on MacConkey agar for E. coli. Dishes were incubated overnight at 35 ºC. Colonies were counted, mean values were compared to the *E.coli* induced group, and % activity was computed. This method was repeated for 48, 72, and 144 hours, respectively, until no colonies grew.

2.14. Statistical Analysis:

To ensure statistical validity, totally experimentations were made in triplicate. The outcomes were presented as mean ± SD. Data sets were examined using two-way ANOVA, through P-values < 0.05 showing significant variances. The examined data/results were given as tables.

3. RESULT

3.1. Plant authentication:

Plant was authenticated by N.K. Dubey department of botany B.H.U., Varanasi specimen no. *Phyllantha.* 2026/01.

3.2.Cultural characteristic:

Urine specimens were cultured on MacConkey agar, and ten isolates were identified as lactose fermenters based on the development of pink-coloured colonies. These cultural characteristics were found to be consistent with the reported features of *Escherichia coli*.

3.3. Microscopical examination and biochemical characteristics:

The purified colonies were characterized based on their Gram-staining reactions and microscopic morphology. The bacterial isolates were observed as Gram-negative, rod-shaped, non-spore-forming bacteria with peritrichous flagella. Based on these microscopic characteristics, the suspected Escherichia coli isolates were further evaluated using biochemical tests. As presented in Table 1, all ten isolates showed positive reactions for catalase, indole, methyl red, and lactose fermentation, whereas negative reactions were observed for oxidase, citrate utilization, and Voges–Proskauer tests. These findings were consistent with the characteristics.

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Table no 3. Screening of *E. coli*:

Biochemical	Isolated									
	E6	E7	E8	E9	E11	E15	E16	E17	E18	E21
Catalase test	+	+	+	+	+	+	+	+	+	+
Oxidase test	-	-	-	-	-	-	-	-	-	-
Indole test	+	+	+	+	+	+	+	+	+	+
Methyl-red test	+	+	+	+	+	+	+	+	+	+
Vogas-Proskauer test	-	-	-	-	-	-	-	-	-	-

3.4. Preliminary phytochemical examination:

Leaves extract of *Phyllanthus niruri* revealed the occurrence of flavonoids, tannins, saponins, steroid and triterpenoid improved antibacterial action. Phyllanthus niruri Leaves hydro-methanolic extract produced Percentage Yield of Extract 500 g of leaves coarse powder of *P. niruri* provides 60 g of excerpt, then the yield is 12%.

Table no. 4. Preliminary phytochemical investigation

Phytochemicals	Name of the test	Observation	Result
Alkaloids	Mayer's Wagner's Hager's	Milky white ppt. Deep chocolate ppt. Yellow crystalline ppt.	Absent Present Present
Flavonoids	Shinoda Specific Lead acetate	Pink pigment Orange to red Yellow tinted	Present Present Present
Proteins and Amino acids	Biuret Millon's Ninhydrin	No ppt No ppt No ppt	Absent Absent Absent
Glycosides	General test Legal′s test Baljet test	No ppt No ppt No ppt	Absent Absent Absent
Triterpenoid	Salkowski's test	Grow Golden yellow layer	Present
Tannins	Gelatin test	Improve white precipitous	Present
Steroid	Libermann-Burchard's test	Light green color	Present
Saponins	Foam test	Foam produced	Present

3.5. *In-vitro* antibacterial action of hydro-alcoholic leave excerpt of *Phyllanthus niruri* against *Eschrerichia coli,* zone of inhibition (mm):

Table no. 5. *Phyllanthus niruri* against *Eschrerichia coli,* zone of inhibition (mm):

Sr. No.	Treatment Groups	Zone Of Inhibition (mm)
1	Test-1 HMEPN (25mg/ml)	16.4±12
2	Test-2 HMEPN (50mg/ml)	18.5±38
3	Test-3 HMEPN (100mg/ml)	21.2 ± 17^{T}
4	Test-4 HMEPN (200mg/ml)	$22.6\pm43^{\neq}$
5	Control Nitrofurantoin (50ug/ml)	25.4±22*

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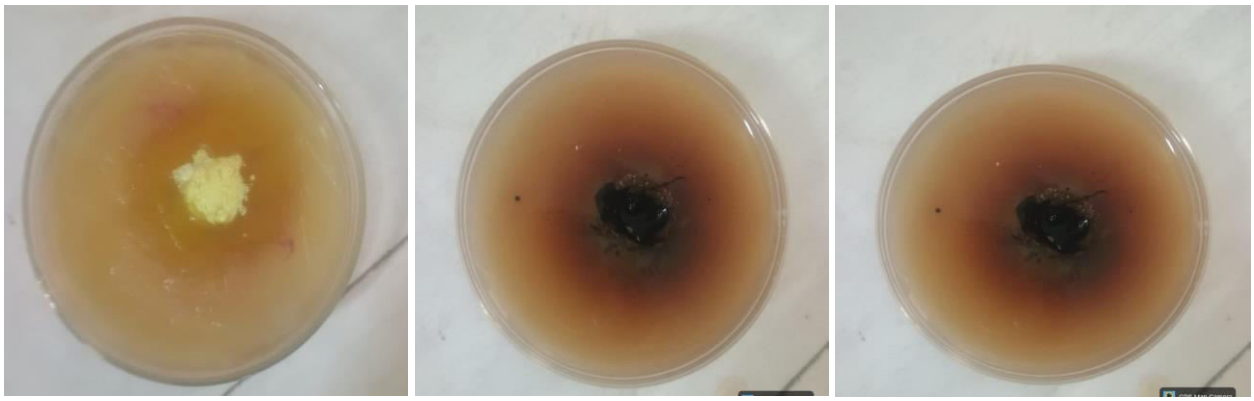
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|www.ijirset.com |A Monthly, Peer Reviewed & Refereed Journal| e-ISSN: 2319-8753| p-ISSN: 2347-6710|

Volume 15, Issue 10, October 2026

|DOI: 10.15680/IJIRSET.2026.1510104|

*P<0.05 when matched to control (Nitrofurantoin)
TP<0.05 when matched to 100mg/ml concentration.
≠P<0.05 when matched to 200mg/ml concentration



A. Nitrofurantoin (50ug/ml) B. Test-3 *HMEPN*(100mg/ml) C. Test-4 *HMEPN* (200mg/ml)

Figure no. 3. Zone of inhibition (mm) of different drugs:

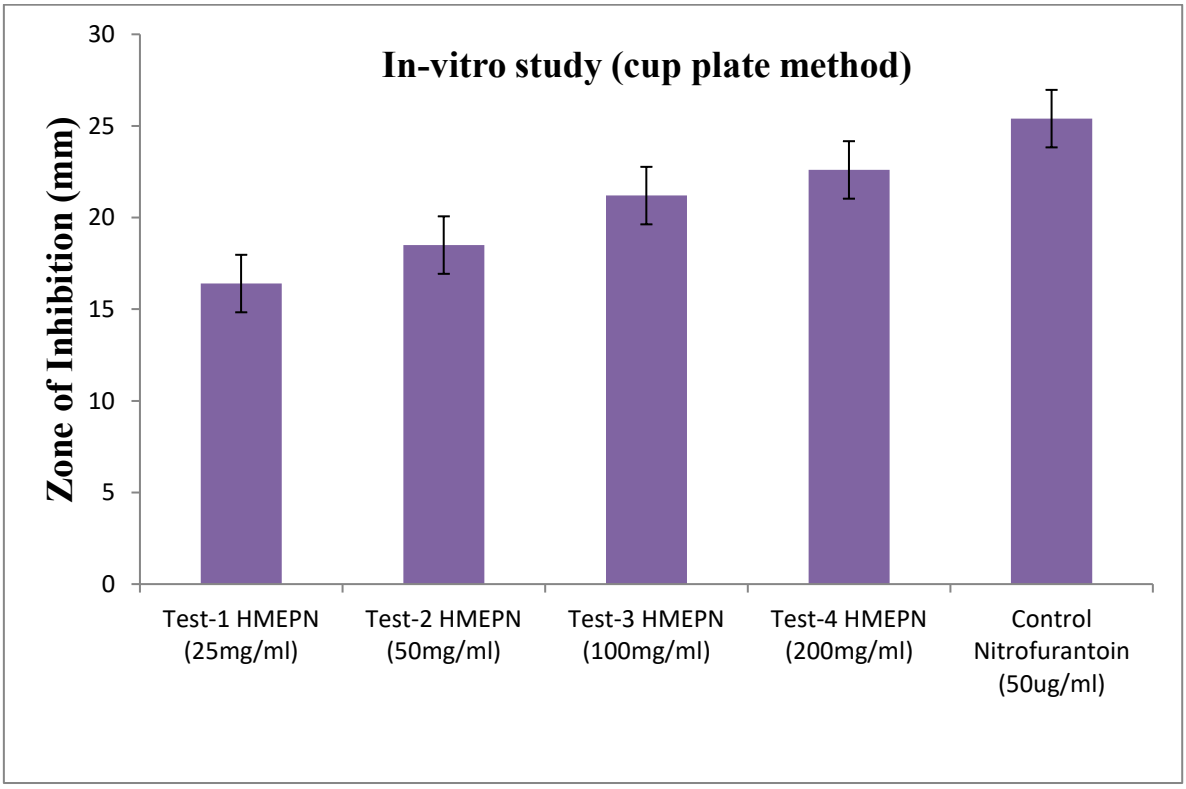


Figure no. 4. Hydro-alcoholic leave extract of *Phyllanthus niruri* against *Eschrerichia coli,* zone of inhibition (mm):

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3.6. MIC of hydro-alcohlic excerpts of *Phyllanthus niruri* against *Eschrerichia coli:*

Table no. 6. MIC of hydro-alcohlic excerpts of ***Phyllanthus niruri*** against ***Eschrerichia coli***

Sr. No.	Dosages of extracts (mg/ml)	Minimum inhibitory concentration (MIC)
1	200	-
2	100	-
3	50	+
4	25	+
5	12.5	+
6	6.25	+
7	3.125	+
8	Nitrofurantoin (50ug/ml)	-

Key: —: No growth, +: Growth

Table no.7. Minimum bactericidal concentrations (MBC) of hydro-alcohlic extracts of ***Phyllanthus niruri*** against ***Eschrerichia coli:***

Sr. No.	Concentrations of extracts (mg/ml)	Minimum bactericidal concentrations (mg/ml)
1	200	-
2	100	-
3	50	-
4	25	+
5	12.5	+
6	6.25	+
7	3.125	NT
8	Nitrofurantoin (50ug/ml)	-

Key: —: No growth, +: Growth, NT: Not tested

Table no. 8. Colony growth of hydro-alcohlic extracts of ***Phyllanthus niruri*** against ***Eschrerichia coli:***

Sr. No.	Concentrations of extracts (mg/ml)	Colony growth		
		48 Hours	72 Hours	114 hours
1	200	-	-	-
2	100	-	-	-
3	50	+	+	-
4	25	+	+	+
5	12.5	+	+	+
6	6.25	NT	NT	NT
7	3.125	NT	NT	NT
8	Nitrofurantoin (50ug/ml)	-	-	-

Key: —: No growth, +: Growth, NT: Not tested

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IV. DISCUSSION

In vitro, the leaf extract showed better effectiveness against E. coli at concentrations of 25, 50, 100, and 200mg/ml. However, the leaf excerpt at 25mg/ml displayed less activity than the 200mg/ml dose. The in vitro and in vivo bacterial protective studies revealed that 200mg/ml leaf excerpts showed much higher antibacterial activity than the 25mg/ml extract. This might be due to the occurrence of an active chemical, which may be more concentrated in the leaf. The study found no substantial variance between the 200mg/ml extract and the control at $P < 0.05$. Additionally, the extract prevented the development of *E. coli* significantly. The high ZOI revealed by the leaf extracts on E. coli suggests that the plant can be utilized to treat infectious disorders caused mostly by microbes. The dose of 200mg/ml had the greatest ZOI for E. coli, indicating that as the dose of the extract increased the antibacterial action. Tables 8.2 and 8.3 show that ZOI and (MIC) of plant extracts (leave). For *Eschrerichia coli,* the minimum inhibitory concentration of the leave excerpts was 100 mg/mL. The in vivo assay revealed that the leaf extract 200mg/kg was more active than the 100mg/kg. The dosage of 200mg/kg had the greatest colony decrease for E. coli, indicating that as the dosage of the excerpts was raised, the number of colonies decreased till there was a clear dish, but there was no significant variance when dosages were related, which could be attributed to the fact that antibacterial activity of a substance is dependent on the active component reaching an organism. When matched to the positive control, there was no substantial variance between the cure groups; this may indicate that the plant excerpt has antibacterial action and might be used to treat infectious disorders.

In the similar vein, when cure groups were matched to the negative control, there was a substantial variance, advising these plant excerpts may have antibacterial properties. The antibacterial screening of crude hydro-alcoholic extract *in vivo* indicated antibacterial activity against E. coli at concentrations of 100mg/kg and 200mg/kg. After 114 hours of treatment, the extracts had entirely suppressed the bacterial colony.

V. CONCLUSSION

Phyllanthus niruri's antibacterial activity in this study support its usage in hydro-alcoholic extract therapy of infectious illnesses. This study and earlier findings demonstrate that *Phyllanthus niruri* has excellent antibacterial properties in vitro. The in vivo investigation originate that *Phyllanthus niruri* excerpts had good antibacterial action against Gram-positive and Gram-negative *(E. coli)* pathogens. It demonstrates these plant excerpts have effect against Gram-negative germs. This study found that the plant excerpt leaf included phytochemical elements of pharmacological value, shown excellent antibacterial activity in vitro and in vivo on the experimental animal.

Future prospects:
Despite this, *Phyllanthus niruri's* leaves have been used as antibacterial activity. The present study therefore set out to assess the antibacterial properties of the leave extract of *Phyllanthus niruri's*.in an experimental animal model and contrast such properties with those of the standard medication, Nitrofurantoin.

VI. ACKNOWLEDGMENT

The authors would like to thanks Dr. Abhay Pratap Yadav for permitting permission to use the institute facilities to carry out my research work. Our sincerely thanks to my guide Mrs. Bhavana Yadav for her guidance and care throughout the work.

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International Journal of Innovative Research of Science, Engineering and Technology (IJIRSET)

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