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IN-VITRO ON PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *PHYLLANTHUS NIRURI* LEAVES EXTRACT

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Abstract

The medicinal plants represent an enormous reservoir of potential phytochemical compounds that could be useful as an alternative to allopathic drugs and are being used to develop Pharma drugs. *Phyllanthus niruri* has medicinal properties for the effective management of several aliments including Hepatitis. The present investigation was aimed to focus on the screening of phytochemical constituents. And additionally antibacterial activity of phyllanthus and antibacterial activity of Phyllanthus niruri in ethanolic and methanolic extracts. The main objective of the present work to established the preliminary phytochemical analysis and *in vitro* antibacterial activity of methanol and ethanol extract of *Phyllanthus niruri.* Furthermore, the presence of these phytochemicals in *Phyllanthus niruri* can act as the therapeutic agents and they are responsible for antibacterial activity.

Keywords: Phytochemicals, Phyllanthus niruri, DMSO.

1 Introduction

Nowadays plants have been recognized as a great source in herbal medicine, complementary pharmaceutical products and leading for new drugs design. Medicinal plants are the indispensible reservoirs of many chemical compounds either primary or secondary metabolites [1]. Plants' worth is something we are well-informed about. There is an abundance of possible pharmaceuticals in the plant kingdom, and the importance of medicinal plants has been better recognized in recent years. There are less side effects, fewer costs, and more accessibility with plant-based medications. When looking at the current search for therapeutically effective new medicines including anti-cancer agents, antibacterial pharmaceuticals, and antimicrobial compounds, plants that have been used medicinally for thousands of years seem to be the best option. Medicinal plants are the most reliable source for many different types of drugs, says the World Health Organization (WHO). Traditional medicines, which are used by around 80% of the population in developed countries, sometimes contain ingredients made from plants that have therapeutic properties. But studies on these plants are needed to learn more about their characteristics, security, and effectiveness [2]. What we call "phytochemicals" are actually substances that plants produce. This is something that the plant's primary and secondary metabolisms produce. Plants can't stay alive or fight off predators like animals, insects, and microbes without these phytochemicals [3]. Additionally, they shield plants from pollution, ultraviolet light, stress, and dehydration, among other environmental hazards. From ancient times, they have been used as both traditional medicine and poisons [4]. Over eighty percent of the population in developing nations uses traditional remedies made from plants as their main source of medical treatment, according to studies conducted by the World Health Organization (WHO). "Phytomedicines serve as a bridge between traditional and modern medicine." Medicinal plants are an integral part of people's and communities' health in many developing countries. Modern-medical preparations rely on traditional medicines. Traditional medicine relies on medicinal plants, which are supposedly safer, to treat a wide range of illnesses [5]. Proteins, carbohydrates, lipids, vitamins, and minerals are some of the most essential building blocks for animal and human development that are found in plants. Most phytochemicals fall into one of two categories: main metabolites or secondary metabolites. Essential for the plant's basic development are sugars, proteins, amino acids, nucleic acids, chlorophyll, and other basic metabolites [6]. In harsh conditions, plants rely on secondary metabolites to stay alive. Flavonoids, tannins, saponins, alkaloids, steroids, and phytosterols are just a few examples of the secondary metabolites that have been shown to have a wide range of commercial uses, including as flavoring agents, pesticides, insecticides, coloring agents, medicines, and antibacterial

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and antifungal products [7]. In addition, they can be used to prevent a wide range of diseases and conditions, including cancer, lung cancer, neuronal disorders, diabetes, heart disease, arthritis, and aging, to name a few [8]. From the very beginning of human history, phytomedicines have made use of plant-based remedies. Make it with anything you have on hand: bark, leaves, flowers, roots, fruits, seeds, etc. Acquiring an understanding of the chemical components found in plants is highly beneficial as it will aid in the creation of intricate chemical compounds [9]. The primary objective of our study was to identify any phytochemicals in the Phyllanthus niruri plants collected in the Tirupattur area of Tamil Nadu, India, and to determine whether or not these plants have antibacterial properties.

2 Experimental

2.1 Collection of *Phyllanthus niruri*

The selected *Phyllanthus niruri* were purchased from local market in Tirupattur, dried and converted into a powder using an electric blender. After the dried powder were used for further analysis [10]. The Fig. 1. Shows the collection and preparation of *Phyllanthus niruri.*

Fig.1. Collection and preparation of Phyllanthus niruri.

2.2Methodology of Extraction of Phyllanthus niruri

The preferred 5 grams of plant powder were dissolved in 50 ml of methanol, and then the same amount of powder was dissolved in 50 ml of ethanol. Each of these solutions was independently extracted using the Soxhlet equipment. Whatman No. 1 filter paper was then used to filter these extracts. A rotatory evaporator was subsequently used to concentrate the filtrates [11-13].

The concentrated extracts were then allowed to dry completely for two or three days at room temperature. After the botanical extracts had dried, they were stored in sterile bottles until needed again.

2.3. Preliminary Phytochemical Screening

The methanolic and ethanolic extract of Phyllanthus niruri solutions were assessed for the existence of the phytochemical analysis by using the following standard methods [6].

1. Detection of Alkaloids

Mayer's test

The extract underwent treatment with Mayer's reagent. The presence of alkaloids is indicated by the formation of a yellow cream precipitate.

Wagner s test

The extract was treated with Wagner s reagent. Formation of brown/reddish brown precipitate indicates the presence of alkaloids.

2. Detection of Flavonoids

Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Sulphuric acid test

Extracts were treated with few drops of H_2SO_4. Formation of orange colour indicates the presence of flavonoids.

3. Detection of Steroids

Two ml of acetic anhydride was added to five ml of the extract and then added each with two ml of H_2SO_4. The color was changed from violet to blue or green indicates the presence of steroids

4. Detection of Terpenoids

Salkowski s Test

The extract was combined with chloroform at a ratio of 5 ml to 2 ml, and then 3 ml of concentrated H_2SO_4 was added slowly to create a distinct layer. The presence of terpenoids is indicated by a reddish-brown color on the inner surface.

5. Detection of Phenols

Ferric chloride test

10ml of the extract was treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

Lead acetate test

10 ml of the extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

6. Detection of Saponins

About 0.5ml of the extracts was shaken with five ml of distilled water. Formation of frothing (appearance of creamy of small bubbles) shows the presence of saponins.

7. Detection of Tannins

After dissolving a little amount of extract in water, it was heated on a water bath. Following filtering, ferric chloride was added to the resulting mixture. The result was a shade of dark green. The presence of tannins is indicated by it.

8. Detection of Carbohydrates

0.5ml extracts were dissolved individually in five ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

2.4 Anti-bacterial activity of Phyllanthus niruri

Phyllanthus niruri extracts prepared from ethanol and methanol were examined for their antibacterial capabilities. Four distinct bacterial strains were used to assess Phyllanthus niruri's antibacterial capabilities. To prepare this nutrition agar medium, 2.5 grams of nutrient agar were mixed with 100 milliliters of distilled water and autoclaved at 37 degrees Celsius for 24 hours. Apply the bacteria to the petri dish by means of the inoculation wire loop. The discs were made using grade 1 filter paper, which is the maximum level of purity. On infected agar plates, the discs were washed with 10, 20, and 30% w/v Phyllanthus niruri extracts. The next step was to incubate the plates at 37 degrees Celsius for a whole day. The antibacterial activity was determined by comparing the zone of inhibition to that of streptomycin, a commercial antibiotic, which served as a positive control. We tested every single extract and every single dose that could be administered [14-15].

3 Results and Discussion

3.1. The Preliminary Phytochemical Analysis of methanolic and ethanolic extract of Phyllanthus niruri

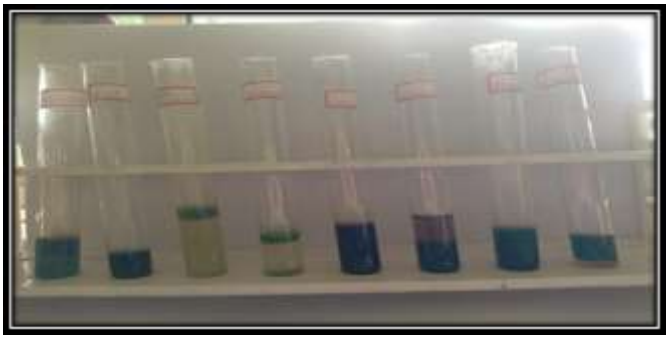
The preliminary phytochemical screening of methanolic and ethanolic extract of plant source showed the presence of carbohydrates, flavonoids, steroids, terpenoids, tannins, quinones and the absence of phenols saponins and glycosides were respectively [16]. From fig 2 shows a

Table 1: The Preliminary Phytochemical Analysis

Phytochemical Constituents	Extraction of *Phyllanthus niruri*		
	Methanol	Methanol + Overnight soaking	Ethanol
Carbohydrates	+	+	+
Alkaloids	+	+	+
Flavonoids	+	+	+
Steroids	-	-	-
Terpenoids	+	+	+
Tannins	+	+	+
Quinones	+	+	-
Saponins	+	+	+
Glycosides	+	+	+
Phenols	+	+	+

Indicates: + Present and – Absent

qualitative phytochemical analysis was conducted on the methanolic and ethanolic extracts of Phyllanthus niruri leaf. The extracts were obtained by soaking the leaf in methanol overnight. The purpose of the analysis was to identify the presence of various phytochemicals, including carbohydrates, phenols, flavonoids, quinones, steroids, tannins, saponins, terpenoids, and alkaloids. The outcomes are presented in Table 1. The methanol leaf extract contained carbohydrates, tannins, alkaloids, flavonoids, phenols, terpenoids, saponins, glycosides, and quinones. The methanol extract is devoid of steroids. The ethanolic leaf extract contains carbohydrates, tannins, saponins, alkaloids, quinones, phenols, terpenoids, flavonoids, and glycosides. The ethanol leaf extract did not include steroids [17-18]. The Table 1 shows the Preliminary Phytochemical Analysis methanolic and ethanolic extract of Phyllanthus niruri as follows, the findings indicate that the methanol and ethanolic leaf extracts of the researched plant were examined. The methanol leaf extract has a greater number of phytochemical elements compared to the ethanol extract (fig 3).



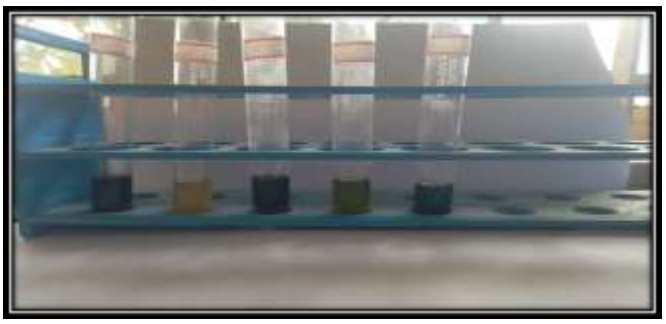
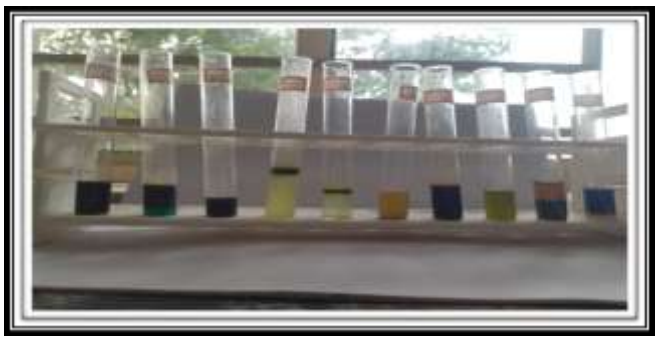


Fig.2. Ethanolic Extraction of *Phyllanthus niruri*



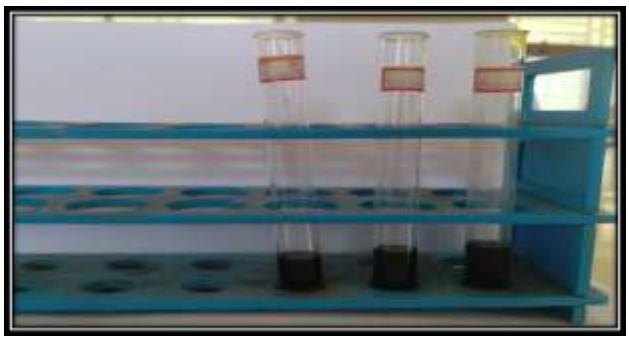
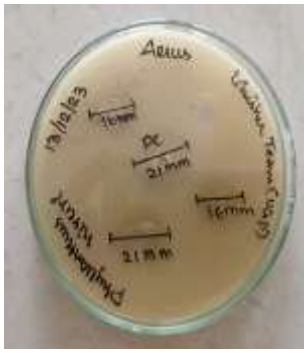


Fig.3. Methanolic Extraction of *Phyllanthus niruri*

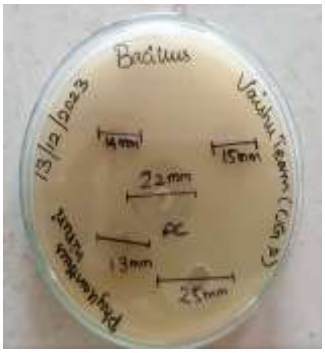
3.2. Anti-bacterial Activity of *Phyllanthus niruri*

Antibacterial activity was assessed using the conventional agar well diffusion method to determine the *Phyllanthus niruri* extracts and herbal beads (Table 2). Using 2% DMSO (dimethyl- sulphoxide) as negative control, same concentrations of the extracts (Extracts 1, 2, 3) were created. By using the spread plate approach, test microorganisms Escherichia coli and Bacillus subtilis were seeded onto the appropriate Mueller-Hinton agar medium. After that, the plate was incubated for 24 hours at 37°C. By measuring the diameter of the inhibitory zone that formed around the well, the antibacterial activity was evaluated [19].

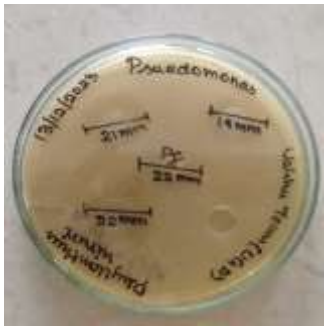
The *Phyllanthus niruri* has carryout Anti- bacterial activity towards the potential of gram positive & gram-negative bacteria for this were used four types of organism's *pseudomonas aeruginosa, Escherichia coli, bacillus subtilis & staphylococcus aureus. Staphylococcus aureus, bacillus subtilis* are gram positive bacteria & pseudomonas aeruginosa, Escherichia coli are gram negative bacteria for this experimental research work [20]. From fig 4 shows that the Phyllanthus niruri had an Anti-bacterial effect agent these four as shown by an evident the zone of inhibition at 3 different concentration the Phyllanthus niruri that has been dissolved in DMSO (6mg/3ml) in quantities of 25,50,100 µg DMSO was used as a negative control the zone of inhibition of Phyllanthus niruri.

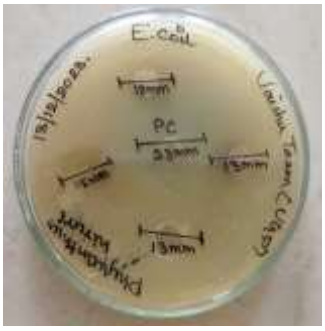


Staphylococcus aureus



Bacillus subtillis





Pseudomonas aeruginosa *Escherichia coli*

Fig.4. Anti-bacterial activity of *Phyllanthus niruri*

Table 2: Anti-bacterial activity of *Phyllanthus niruri*

s. no	Organisms	NC DMSO	PC	Zone of inhibition in 25 µg/ml	Zone of inhibition in 50 µg/ml	Zone of inhibition in100µg/ml
1.	*Pseudomonas aeruginosa*	Nil	(Gen) 2.0 (mm)	2.1	2.5	2.7
2.	*Escherichia coli*	Nil	(Gen) 2.0 (mm)	2.0	2.8	3.1
3.	*Bacillus subtilis*	Nil	(Gen) 3.1(mm)	3.1	3.0	3.1
4.	*Staphylococcus aureus*	Nil	(Gen) 3.2(mm)	2.7	3.0	3.5

4 Conclusions

It can be concluded that the antimicrobial activity and preliminary phytochemical screening were successful in warding off the diseases. Current experimental evidence suggests that plants are rich in phytochemicals with potential medical use in the treatment of a wide range of illnesses. Carbohydrates, proteins, phenols, saponins, glycosides, amino acids, flavanoids, terpenoids, tannins, and, in the lack of steroids, quinones were the phytoconstituents most prevalent in plants. In comparison to the positive control, gentamicin, and tetracycline, the microorganism's *pseudomonas aeruginosa, Escherichia coli, bacillus subtilis, and staphylococcus aureus* showed promising antibacterial activity. To identify the active ingredient or compounds

responsible for the antibacterial activity, further research on P. niruri is necessary.

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Conflict of Interest: Nil

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