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Ethanolic, Methanolic, and Aqueous Extracts of Phyllanthus Niruri to Look at Its Antibacterial Properties and Phytochemicals

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Abstract: ***As a possible addition to traditional medicine, pharmaceutical companies are turning more and more to the large number of phytochemical compounds found in medicinal plants. These chemicals are also used to make drugs. Because of its healing properties, Phyllanthus niruri has been shown to help treat a number of diseases, such as hepatitis. The main goal of this study is to find out what phytochemical compounds might be present.***
The goal of this study was to look at the phytochemical makeup and antibacterial activity of Phyllanthus and Phyllanthus niruri extracts in water, ethanol, and methanol. There are many different compounds in PN. Some of them are saponins, tannins, flavonoids, steroids, and cardiac glycosides. Different concentrations of methanol, ethanol, and water extracts were tested to see if they could kill bacteria. When compared to the other extracts, the 30% weight-per-volume methanolic extract had the largest zone of inhibition.
Each extract of P. niruri has secondary metabolites that could be used in medicine and act as antibacterial agents against a wide range of bacteria. These PN phytochemicals have properties that kill bacteria and get rid of free radicals, and they may be used in therapeutic interventions.
Keywords: Ethanolic, Methanolic, Aqueous, Antibacterial activity, Phyllanthus niruri, Phytochemical

I. INTRODUCTION

The world of plants is full of potential medicines, and in recent years, it has become clearer that plants can be used as a source of medicine. Medicines made from plants are easy to get, cheap, safe, effective, and rarely cause unwanted side effects. Before bioactive chemicals can be isolated, phytochemical screening must be done.

This helps find the most important chemical parts of the plant. Scientists have been able to isolate and identify a wide range of bioactive phytoconstituents since the middle of the 19th century. Most people agreed that, in addition to their physiological role, they also had therapeutic value.

Plant-based biomolecules have been shown to have a solid scientific basis, and they may one day replace antibiotics as a treatment option for a wide range of bacterial infections and human diseases. Many of them are used today as either the main or secondary ingredients in many medicines.

The tiny Phyllanthus niruri L. (Euphorbiaceae) plant can be found in most tropical and subtropical places. This plant is called "dukong anak" in its native language, which means "child's ear." Since more than 2,000 years ago, P. Niruri has been used in Ayurvedic medicine to treat jaundice and gonorrhea. Herbalists also use it to treat kidney stones, viruses, germs, diabetes, and a wide range of other conditions.

This plant has been used in the past to treat digestive problems, cough, fever, malaria, hypoglycemia4, cancer5, lowering cholesterol6, and the hepatitis B virus, among other things.

Here, ethanol, methanol, and water were used as solvents to study the antibacterial properties and phytochemical makeup of Phyllanthus niruri. Phytochemicals are groups of chemicals that are found in plants. Antimicrobial treatment gets rid of microorganisms.

II. THE MATERIALS AND METHODS

A. Extraction using Solvents

All the plant parts were spread out in the sun and left to dry in the air until all the water had been taken up by the air and the plants were dry enough to be ground.

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After the plants were dried, they were turned into a powder in a machine blender. After the powder was weighed, measured, and labeled, it was put into containers that kept out air so it could be used later. We made extracts of ethanol, methanol, and water by soaking 100 grams of powdered plant material in 500 milliliters of different solvents at room temperature for 72 hours. This was done to make it possible to make the extracts. The extracts were found after being strained through muslin cloth and what is often called "filter paper for men" (Grade 1). A rotary evaporator is put in a water bath so that the extracts can be made more concentrated. Only about 5 to 6 percent of the plant is taken out.

B. Examination of Phytochemicals

Here, the gold standard of methods and solvents are used to get phytochemicals out of Phyllanthus niruri (ethanol, methanol, and water).

These solvents were made from the Phyllanthus niruri plant.

1) *The Alkaloids Examine (using Wagner's Reagent):* Wagner's reagent, which is 1.27 grams of iodine and 2 grams of potassium iodide dissolved in 100 milliliters of water, was used to make a part of the extract turn a reddish brown color or solidify.
2) *Examine for Terpenoids (Salkowski Test):* We added half a gram of each extract to two milliliters of chloroform. When 3 ml of strong sulphuric acid was added, a layer formed. The rusty brownish red color of the interface can be used to tell if terpenoids are present.
3) *Examine for Tannins:* A half-gram of extract was cooked in a milliliter and a tenth of a liter of water in a test tube, and then the water was filtered. A few drops of 0.1% ferric chloride changed the color from brownish green to blue-black.
4) *Examine for Traces of Saponins (Foam Test):* Two milliliters of extract and two milliliters of water were mixed in a test tube that held six milliliters. The presence of saponins could be checked by shaking the mixture hard and then looking for foam that stays after the shaking stops.
5) *Examine for Quinones:* When the extract was mixed with concentrated HCL, a yellow precipitate was made (or coloration).
6) *Examine for Cardiac Glycosides (Keller Kelliani's Test):* Five milliliters of each extract, two milliliters of glacial acetic acid, and one drop of ferric chloride solution were put into a test tube. After that, one milliliter of sulfuric acid was carefully put on. Deoxysugar was visible as a brown ring around the contact. It is a part of cardiac glycosides. Under it, a violet ring may form, and in the acetic acid layer, a greenish ring may form.
7) *Examine for Phenols (Ferric Chloride Test):* After treating a small number of extracts with an aqueous solution that had 5% ferric chloride, they were looked at to see if they turned dark blue or black.
8) *Examine for reducing sugars (Fehling's Test):* After heating up Fehling's solutions A and B in a test tube, we added the aqueous ethanol extract, which was 0.5 grams of ethanol diluted in 5 milliliters of water. A close look was kept on the solution to see if the color changed.
9) *Examine for Flavonoids:* A few drops of a 10% concentration ferric chloride solution were added to the dissolved extract. You can tell if a phenolic nucleus is there by whether it is green or blue.
10) *Examine for Resins8:* Petroleum ether extract was put into a microcentrifuge tube that held 10 milliliters. Next, we added an equal amount of copper acetate solution to the tube, gave it a good shake, and then let the contents settle. Researchers thought that there were resins because the color was green.
11) *Sterols and Steroids (Salkowski's Examine):* When two milliliters of chloroform were used to treat an extract, the top layer turned a beautiful shade of scarlet, which is a sign that sterols and steroids were present.

C. Evaluation of the Phyllanthus niruri Extract's Antibacterial Activity

The antibacterial properties of Phyllanthus niruri extracts made from methanol, ethanol, and water were tested. The antimicrobial properties of Phyllanthus niruri were tested with five different types of bacteria.

This nutrient agar medium was made by putting 2.5 grams of nutrient agar in 100 milliliters of distilled water and autoclaving it at 37 degrees Celsius for 24 hours. Put the germs on the agar plate using the inoculation wire loop. Filter paper with the highest level of cleanliness was used to make the discs (grade 1). The discs were washed in 10, 20, and 30% w/v extracts of Phyllanthus niruri in methanol, ethanol, and water, and then put on infected agar plates. After that, the plates were put in an incubator at 37 C for 24 hours.

To figure out how much antimicrobial activity there was, the zone of inhibition was compared to that of the commercial antibiotic streptomycin, which was used as a positive control. All of the extracts and all of the possible doses were put to the test.

Table 1: Evaluation of the phytochemical potential of P.niruri in several solvent extracts

Phytochemicals	Ethanolic Extract	Methanolic Extract	Aqueous Extract
Phenolic Compounds	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Alkaloids	+	+	+
Tannins	+	+	+
Cardio glycosides	+	+	+
Steroids	+	+	+
Reducing Sugars	+	+	+
Anthraquinones	+	–	–
Resins	+	+	–
'+' Present; '-' Absent			

III. RESULTS

Alkaloids, terpenoids, tannins, saponins, quinones, cardiac glycosides, phenols, reducing sugars, flavonoids, resins, steroids, and anthraquinones were among the active phytochemicals found in the ethanolic extract of P. niruri. The methanolic extract, on the other hand, shows all of the phytochemicals except the anthraquinones. The aqueous extract, on the other hand, doesn't have any resins or anthraquinones. These phytochemicals have important effects on living things. Several different tests have shown that these metabolites are effective against bacteria. (Table 1). With the agar diffusion method, PN was tested to see how well it killed bacteria from five different human pathogens: Staphylococcus sp., Escherichia coli, Pseudomonas aeroginosa, Bacillus subtilis, and Bacillus cereus. Table 2 shows the results of antibiotic tests done on five different diseases with drugs like streptomycin, which can be bought. Extracts from PN were effective against a panel of potentially dangerous pathogens in a wide range of ways.

When the concentration of the extracts was raised, it was seen that the size of the zone of inhibition gradually grew in all of the tested strains of bacteria. Here are the zones of inhibition of PN against Staphylococcus sp., Escherichia coli, Pseudomonas aeroginosa, Bacillus subtilis, and Bacillus cereus.

Table 2: Antibacterial activity of Phyllanthus niruri in a range of concentrations and solvent extracts

Bacterial species	Zone of inhibition in millimeters (mm)											
	Methanolic extract(W/V)				Ethanolic extract(W/V)				Aqueous extract(W/V)			
	S. *mycin*	30%	20%	10%	S. mycin	30%	20%	10%	S. mycin	30%	20%	10%
Escherichia coli	24	16	12	8	22	11	6	NA	20	12	8	4
Staphylococcus aureus	28	14	12	10	24	09	8	3	26	14	6	NA
Bacillus subtilis	20	17	10	9	22	13	9	8	20	13	9	5
Pseudomonas aeroginosa	28	15	12	7	24	06	4	NA	23	13	4	NA
Bacillus cereus	31	17	13	10	27	07	3	NA	23	8	3	NA

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The values are determined by three distinct analyses; The zone of inhibition is measured in millimeters, excluding the disc's diameter; NA stands for "no action."

In order to study plants as possible sources of new medicines to treat cancer, AIDS, diabetes, Parkinson's disease, and malaria, scientists have to look hard for resources. The discovery of phytochemicals, such as those with cytotoxic and/or anti-tumor effects, could lead to new treatments for a wide range of diseases. To get plant extracts with lots of phytochemicals, you need to come up with good ways to extract them.

The most likely secondary metabolites of PN have been found through a study of its phytochemistry. These include alkaloids, flavonoids, phenol, proteins, amino acids, tannin, and carbohydrates. These secondary metabolites are found when PN is analyzed from a phytochemical point of view. Most people agreed that, in addition to their physiological role, they also had therapeutic value. Because of their connection to cancer activity, phenol components are interesting and important in the field of pharmacy. There are large amounts of alkaloid, tannin, terpenoids, flavonoids, and phenol in samples of ethanol. The phytochemicals in Phyllanthus niruri were tested with ethanol, and all of the bacterial cultures used in this study showed that it worked. Compared to the ethanolic and aqueous extracts, the methanolic extract had the largest amount of zone of inhibition. This could be because PN has chemicals called phenolics, alkaloids, and tannins.

IV. DISCUSSION

Even though modern medicine and pharmaceutical research have come a long way, people have always used plants as medicine. India has about 3,000 plant species that have been shown to have healing properties. As far back as 3700 B.C., the ancient text called the Rigveda talks about how many plants can heal. Herbal remedies were used in many ancient healing practices, like Ayurveda, Yunani, Siddha, and Homeopathy. People think that 40% of the world's population only uses natural remedies made from plants.

The goal of this study was to find out which phytochemicals can be found in PN extracts made with methanol, ethanol, and water. Table 1 shows the results of the phytochemical screening done by PN. Both the ethanolic and methanolic extracts of PN are full of phenolic compounds, saponins, flavonoids, terpenoids, alkaloids, tannins, cardiac glycosides, steroids, reducing sugar, resins, and anthraquinones, but the methanolic extract doesn't have any anthraquinones. All of the phytochemicals in the aqueous extract that have these results show that the plant has a lot of different chemical compounds, and any one of them could be to blame for its many pharmacological effects.

People have said that flavonoids, steroids, glycosides, terpenoids, quinines, and alkaloids make up most of a plant's active ingredients, but their actions were not studied here. Also, the PN contains the chemical saponin, which makes red blood cells clump together more. This helps wounds, ulcers, and bleeding stop. Alkaloids have many important uses, such as making pain go away, stopping muscle spasms, and killing bacteria. Some alkaloids are used for other things.

Chemicals that kill bacteria have been found in large amounts in plants that are used in traditional medicine. There is a chance that the plants could be used to make chemotherapeutics. The antimicrobial properties of P. niruri have been studied much less than those of other bacteria.

Table 2 shows the results of a test to see if PN extracts kill germs. Because each PN extract has its own unique mix of ingredients, the therapeutic effects of these extracts will also be different in kind and size. The anti-inflammatory and antibacterial effects of the ethanol extract may be due to the alkaloids, terpenoids, tannins, and steroids that are found in the plant.

Table 2 shows how different P. niruri extracts work against different microorganisms. When compared to ethanolic and aqueous extracts at 30%, 20%, and 10% concentrations, the antibacterial activity of the 30% methanolic extract was the strongest. The pathogenic bacteria that cause many common skin, urinary tract, and digestive diseases were killed by the methanolic extract of P. niruri. Alkaloids, which can be taken from the plant, are thought to be the reason why Phyllanthus niruri is so effective at killing bacteria. The fact that tannins, flavonoids, triterpenoids, glycosides, and lignans are found in the plant extract of Phyllanthus may explain why it kills bacteria. Other possible candidates for this hypothesis are the carotenoids phyllanthin and hypophylanthin.

Because methanol was better at dissolving the antibacterial chemicals in plants, its antibacterial action was stronger and its zone of inhibition was bigger. Since the active chemicals dissolve more easily in methanol, it's possible that this solvent makes the extracts more powerful.

Extracts of water, ethanol, and methanol all stopped the spread of E. coli just as well. The methanolic and aqueous extracts did the best job of stopping S. aureus from growing. Compared to S. aureus, E. coli was much easier for the ethanolic extract to stop from growing than S. aureus. Botanical mixtures that were thought to make people healthier and happier have been used and respected for a long time. Plant extracts that have been shown to kill bacteria could be very useful in medicine.

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V. CONCLUSION

The results of this study could be very useful for measuring the amount of total phenols, tannins, and flavonoids. You can't say enough about how important these results are. Based on these results, it seems that ethanolic, methanolic, and aqueous extracts are used in both traditional medicine and the making of new drugs to get at new bioactive components. Extracts of PN have been shown to kill bacteria in different concentrations in ethanolic, methanolic, and aqueous environments. The next step is to use PN to treat illnesses caused by bacteria.

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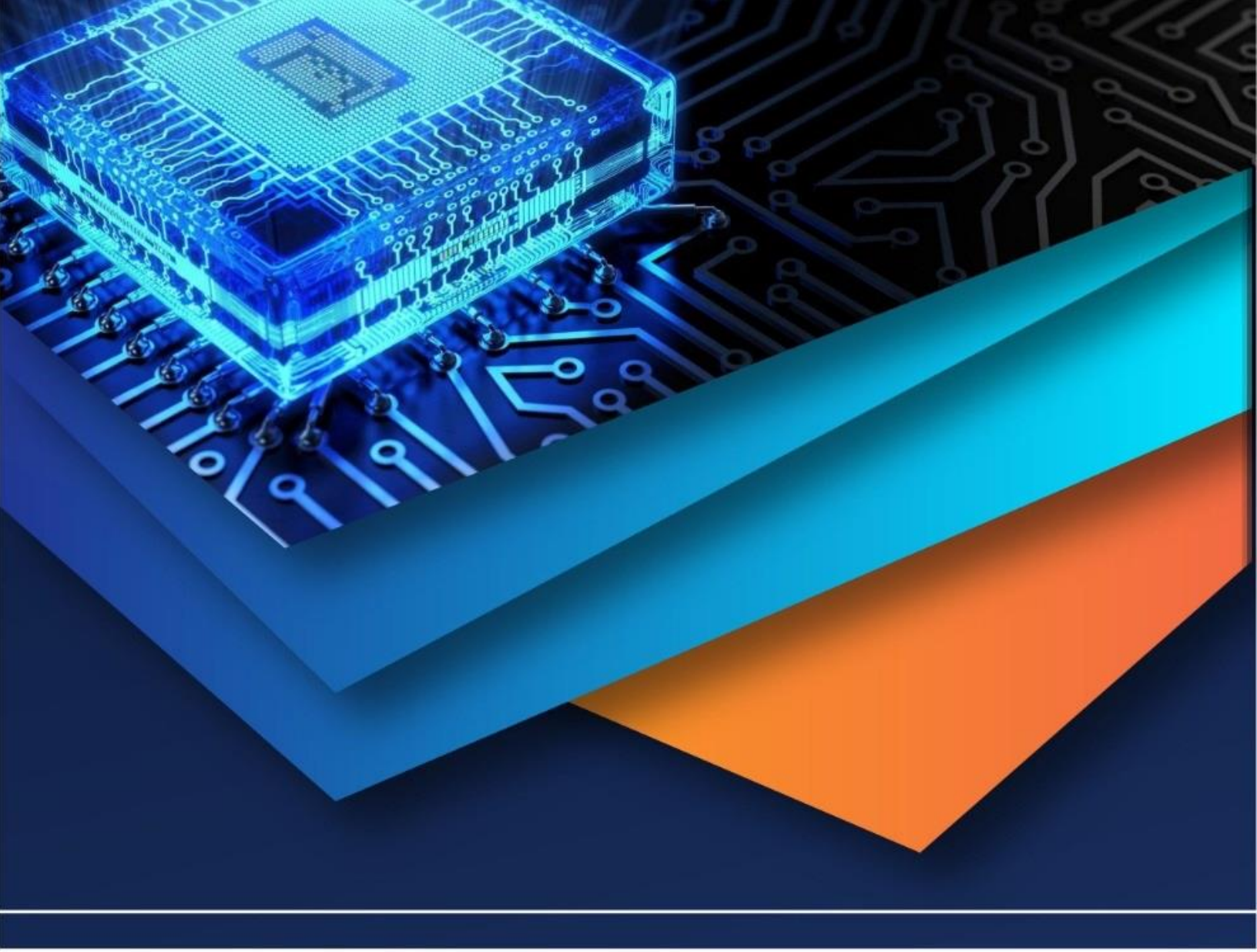

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