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To Study Phytochemical Investigation and Antibacterial Study of Azadirachta Indica against Enterococcus Faecalis

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Abstract

Background and Objectives: *This study looked into the phytochemical components and antibacterial activity of A. indica extracts against E. faecalis, a varied group of facultative anaerobic, gram-positive bacteria that could be used to create new therapeutic approaches.*

Material and Methods: *The phytochemical makeup and antibacterial activity of A. indica against Enterococcus faecalis (E. faecalis) were looked at in this work. The active ingredients from A. indica leaves were taken out using both water and ethanol as solvents. We used the disc diffusion method to test how well both A. indica extracts killed E. faecalis bacteria at different amounts. The zones of inhibition showed how sensitive the bacteria were.*

Results: *According to this study, both samples had phytochemicals in them, such as tannins and phenolics. In addition, the water-based extract only had alkaloids, flavonoids, and saponins. Also, steroids were only found in the ethanolic extract. The ethanolic and aqueous extracts were both successful against E. faecalis, with 20 mg/mL being the lowest concentration needed to stop growth. As the concentration of the extract went up, the inhibition zones got bigger. The ethanolic extract gave the biggest reading, 9.6 mm. This study shows that both ethanolic and water-based extracts of A. indica are very good at killing germs because they stop E. faecalis from growing.*

Conclusion: *According to these results, neem could be used to make new medicines, and they also show that it has been used traditionally as an antibacterial agent.*

Keywords – *Azadirachta indica, phytochemicals, and antibacterial activity, E. faecalis.*

INTRODUCTION

Enterococci are a normal part of the bacteria of the digestive tract. When broad-spectrum antibiotics are given to sick patients, enterococci are killed. This makes the protective layer of gastrointestinal mucus thinner. This could make it easier for antibiotic-resistant hospital-acquired enterococcal clones to spread without a good reason [1-3]. Enterococcus faecalis and Enterococcus faecium (E. faecium) are the main types of Enterococcus that are harmful to people. About 85–90% of enterococcal illnesses are caused by E. faecalis, and 5–10% are caused by E. faecium. These two species can also cause endocarditis, newborn sepsis, bacterial infections after surgery, and sometimes meningitis [2-4].

Infectious diseases often put people and animals in danger and cause a lot of pain and death around the world. There are a lot of these diseases in developing countries, especially in Africa [3-5]. They are usually caused by infectious microorganisms like bacteria, viruses, parasites, and fungi. Research shows that standard drugs can effectively treat a wide range of illnesses, especially infectious ones. Because of this, traditional medicine and plants are the main ways that more than 80% of the people in sub-Saharan Africa get medical care. Azadirachta indica (A. indica) is a traditional medicine that works well for some health problems [4-6].

The Azadirachta indica (A. indica) neem tree could be used as a medicine, but more research needs to be done before it can be used. Many types of microorganisms have been shown to be susceptible to this plant's antibacterial properties [5-7]. The antibiotic property of A. indica can be used against Gram-positive and Gram-negative bacteria such as Escherichia coli (E. coli) and Enterococcus species. It also works against diseases that are immune to drugs, which makes it a possible antibiotic. Neem, or A. indica (Meliaceae), is a plant that is native to India and grows wild in many tropical and subtropical places. It is used for medicine [6-8].

The phytochemicals in A. indica, which is also known as neem, make it useful as a medicine and have bodily effects on people. Neem has many plant chemicals in it, such as steroids, glycosides, anthraquinones, flavonoids, glycosides, anthraquinones, saponins, and tannic acid. Neem leaf products have many health benefits, such as killing bacteria, fungi, viruses, sugar bugs, mosquitoes, bedbugs, and other bugs, as well as helping the heart, pyorrhea, and scabies [7-9]. Neem extracts from the leaves, roots, bark, and seeds have been shown to be effective at killing E. faecalis and Streptococcus germs. Many types of bacteria, both Gram-positive and Gram-negative, can be killed by neem leaf, seed, and bark oil [8-10].

The aim and objectives of this study was to study phytochemical investigation and antibacterial study of Azadirachta indica against Enterococcus faecalis.

MATERIALS AND METHODS:

This study examined the phytochemical composition and antibacterial efficacy of A. indica against Enterococcus faecalis (E. faecalis). The active compounds from A. indica leaves were extracted using water and ethanol as solvents. We employed the disc diffusion method to evaluate the efficacy of various concentrations of A. indica extracts in eliminating E. faecalis bacteria. The zones of inhibition indicated the sensitivity of the microorganisms.

Preparation Plant Extraction:

We used a knife to cut the *A. indica* leaves off of the stem, rinsed them under running water to get rid of any dirt, and then left them to dry in the shade for 14 days. Using a mortar and pestle, the dried leaves were ground up into a powder that was then sifted to make it uniform in structure. After weighing 50 grams of coarsely ground *A. indica*, we used 750 milliliters of a liquid mix (ethanol/water) to get the active ingredients out. Whitman's best filter paper was used to clean the blend. They were dried in beakers set over a water bath at 60˚C for 12 hours [9-11].

Phytochemical Analysis:

The plant liquids were analyzed for phytochemicals using a method described in previous research. We used methods from earlier research to look for phytochemicals like steroids, terpenoids, alkaloids, flavonoids, phenols, tannins, saponins, and flavonoids [10-12].

Isolate Collection, Culture, and Inoculation:

The *E. faecalis* isolates used in the experiment were grown in the Microbiology Laboratory. Researchers have already found that the *E. faecalis* isolates were collected using standard methods. In a different study, the Petri dishes with *E. faecalis* isolates were kept at 37 °C for 18 to 24 hours. Using direct state suspension and turbidity concepts, we came up with the 0.5 McFarland standards. It was made sure that clear salt was used to level out the turbidity until it met the 0.5 McFarland turbidity standards [13-15]. To finish the process, the solution and 0.5 McFarland turbidity gauges were put next to the light source, with a white background with strong dark lines separating them. To make Mueller-Hinton agar, Petri dishes were used to make sure the agar didn't get too wet or too dry. To separate *E. faecalis* colonies, a clean cotton swab was used. The colonies were then put into Mueller-Hinton agar petri plates. The next step was to spread the *E. faecalis* by swabbing the agar surfaces at a 45-degree angle and then a 90-degree angle. The infected Petri plates were left to sit for 20 minutes to help the inoculum mix with the cells before *A. indica* extracts and ciprofloxacin were added [16-18].

Antimicrobial Activity study:

The disk diffusion method was used to test how well *A. indica* killed *E. coli* germs. It was possible to make *A. indica* extracts in water and alcohol at amounts of 1 mg/mL, 10 mg/mL, 20 mg/mL, 30 mg/mL, 40 mg/mL, and 50 mg/mL from a 50 mg/mL stock solution. For the water extract, clean distilled water was used to thin out the stock solution [19-22]. For the ethanol extract, Dimethyl Sulfoxide was used to do the same thing. The Millipore filter was made germ-free, and the right amount was put on germ-free filter paper plates. The filter paper discs were put on Mueller-Hilton agar plates and cultured for 24 hours at 37°C. The discs were filled with different amounts of plant extracts. A plastic scale was used to measure the inhibition zones in millimeters, and the results were compared to the plant extract amounts that worked. DMSO and clean water were used as negative controls. Ciprofloxacin 5 µg standard discs, on the other hand, were used as positive controls against E. faecalis. In an earlier study, we found the lowest concentration of an inhibitory substance that stopped the growth of *E. faecalis* [23-26].

RESULTS AND DISCUSSION:

Extraction Study:

The process of extracting *A. indica* using ethanol and water is shown in Table 1. The higher percentage of extract made shows that the water extraction method was better at getting important parts from the plant material than the ethanol extraction method.

Table 1: Weight of *A. indica* extract, initial weight, and plant yield (%)

Solvent	**Initial Weight (gm)**	**Weight of extract (gm)**	**Extraction Yield (%)**
Ethanol	60	9.2	18.36%
Water	60	14.12	28.55%

Phytochemical Analysis:

In Table 2, you can see the phytochemical study of *A. indica* extracts in both ethanol and water. The water-based extract was found to contain phytochemicals such as alkaloids, flavonoids, phenolics, saponins, and tannins. The ethanolic extract still had phenolics and tannins, but it didn't have any flavonoids, saponins, or alkaloids (Table 2).

Table 2: Results from a phytochemical study of an *A. indica* extract

Sr. No.	**Phytoconstituents**	**Ethanol extract**	**Aqueous extract**
1	Phenolics	+	+
2	Tannins	+	+
3	Flavonoids	-	+
4	Alkaloids	-	+
5	Steroids	+	-

6	Terpenoids	-	-
7	Saponins	-	+

+ Present, - Absent

***A. indica* aqueous and ethanolic extracts inhibit *E. faecalis*:**
Table 3 shows that the aqueous and ethanolic extracts of *A. indica* are successful at killing *E. faecalis* bacteria. At low quantities, neither the ethanolic extract nor the water extract stopped the growth of *E. faecalis* very much. As the amount of both extracts was increased, their antibacterial activity got stronger until it reached its peak at 50 mg/mL for each. It was found that there was no statistically significant difference between the two extracts when they were at their strongest. Still, Table 3 shows that the amounts of the ethanolic and water-based extracts were different. It was found that ciprofloxacin was a better antibiotic than both the water and alcohol extracts of *A. indica* against *E. faecalis*.

Table 3: Antibacterial activities of *A. indica* extracts

Conc.	**Aqueous extract zone of inhibition (mm)**	**Ethanolic extract zone of inhibition (mm)**	**Mean zone of inhibition (mm) of standard**
1mg/ml	0	0	29 ± 0.0
10mg/ml	0	0	
20mg/ml	7.0 ± 0.82	7.2 ± 1.89	
30mg/ml	8.1 ± 0.33	7.7 ± 2.75	
40mg/ml	9.0 ± 0.86	8.9 ± 0.26	
50mg/ml	9.7 ± 0.82	9.5 ± 1.88	

There are many phytochemicals in *A. indica*, such as flavonoids, phenolics, tannins, saponins, alkaloids, terpenoids, and steroids. We also discovered that *A. indica* had antibacterial activity against *E. faecalis* that depended on the dose, starting at a minimum inhibitory concentration of 20 mg/mL [27-29]. Phytochemicals like flavonoids, phenolics, tannins, saponins, alkaloids, and steroids were found in the *A. indica* extract in this study. The current study found that both the ethanolic and water-based extracts had tannins and phenolics in them. Also, our research showed that the water-based extract had alkaloids, flavonoids, and saponins, but the alcohol-based extract did not. A study done in India also found that the ethanolic extract of *A. indica* contained phenolics and tannins but not saponins. In Ethiopia, Dereje and his colleagues did a study that found terpenoids in both the water and alcohol extracts of *A. indica*. However, the current study did not find any terpenoids in either extract [30-34].

The ethanolic extract of *A. indica* was the only one that showed steroids in our tests. Our results are similar to those of other studies that found steroids and tannins in the ethanolic extract of *A. indica*. The different phytochemical content described may be due to the different polarities of the solvents. Researchers around the world may also find different results about whether the phytochemicals found in this study are present or absent. This is because the plant is affected by its climate and location. There are reports that the antibacterial properties of *A. indica* come from phytochemicals that are effective against a number of diseases [35-43].

CONCLUSION:

The water and alcohol extracts of *A. indica* (neem) were found to have strong antibacterial effects against *E. faecalis* in this study. The effects were concentration-dependent, which shows that these extracts may have antibacterial properties. It seems that neem could be used to make new medicines, and this backs up the idea that it has been used traditionally to kill germs in traditional medicine. There are many phytochemicals in *A. indica* extracts, such as flavonoids, phenolics, tannins, saponins, alkaloids, and steroids. These chemicals give the extracts their complex structure and show that they could be used as new antibacterial agents.

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None

Conflict of Interest

None

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