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In vitro Antimicrobial Effects of *Azadirachta indica* Seed Oil

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ABSTRACT
The study was aimed to determine the antimicrobial activity of *Azadirachtaindica* seed oil against some disease-causing organism such as *Staphylococcus aureus, Salmonella typhi and Pseudomonas aeruginosa.* This study of antibacterial activity against selected pathogens was done by dilution method, agar disc diffusion method and agar well diffusion method. Pathogens were taken from General Hospital Mubi. Locally extracted *Azadirachta indica* oil from tested showed antibacterial activity against all the three pathogenic bacteria even at very low concentration. *Azadirachta indica* seed oil inhibited the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* by 99.99%. The results showed that the oil will be a good source of antibacterial agents. The encouraging observation also indicate that this oil should be exploited as natural antibiotic for the treatment of several infectious diseases caused by these pathogens and could be useful in understanding the relations between traditional cures and current medicines.

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Introduction

Development of resistance by pathogenic microorganisms to drugs is rising great concern in the present era of medical system. Medical xpert reports revealed the increasing inabilities of antibiotics to effectively treat illnesses due to antimicrobial resistance [1, 2]. The Center for Disease Control and Prevention (CDC) in 2013 reported 23 000 deaths and two million illnesses caused by antibiotic resistance in the United States (U.S) costing $20 billion on healthcare [2]. According to literature, by 2050 microbial drugs resistance would result to 10 million deaths worldwide costing $100 trillion on the global economy [2]. This beacons the necessity for finding immediate viable solutions and traditional healing methods using plants and their derivatives have been considered the most reliable resort as they have proven really safe and effective [3]. The use of plants products as a local method of managing illnesses has been in practice dates back from the inception of human civilization and presently, a number of indigenous groups across the world have adopted the use of plants materials to treat assorted comorbidities [4].

As estimated by World Health Organization, nearly 80% developing countries population treat their illnesses traditionally by utilizing medicinal plants. It has also been extrapolated that more than half of the global population still value the traditional way of managing diseases making medicinal plants a highly sorted for medicinal commodities [5]. Medicinal plants contain bio-functional ingredients which makes them exerts widespread pharmacological effects [6]. According to reports, India, Africa, Cuba, Italy, Palestine, Honduras and Jordan, have set in motion systematic research programs to investigate and screen medicinal plants for antimicrobial activities [7]. The antidiabetic potential [8, 9] as well as their effects on some tropical diseases such as malaria, schistosomiasis and leishmaniasis have been observed [9]. Studies have also revealed the antimicrobial possibilities of medicinal plants as they tend to extend the shelf life of foods [10].

Azadirachta indica is a tropical plant from *Meliaceae* plant family. It is popularly known as neem tree in Africa and widely used in India for its many therapeutic applications. Over 135 bioactive compounds have been isolated from different organs of *Azadirachta indica* [11]. Previous studies have revealed its antihyperglycemic activity in type 1 and type 2 diabetes [8, 9]. The antifungal, antiviral and insecticidal activities of *Azadirachta indica* were also observed. Other pharmacological properties displayed by *Azadirachta indica* includes anti-pyretic [12, 13], antimalarial and antitumor [14], anti-ulcer effect [15], anti-fertility effect [16] and antioxidant activity [15]. *Azadirachta indica* (Neem) seed oil contains a diverse range of bioactive compounds responsible for its antimicrobial and therapeutic properties. The oil is particularly rich in limonoids such as azadirachtin and nimbin, alongside fatty acids like oleic and linoleic acids, which enhance its biological activity [17, 18].

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This phytochemical richness supports its increasing application in antimicrobial research and natural health formulations.The aim of this study is to determine the antimicrobial activity of *Azadirachta indica* seed oil on *Salmonella typhi, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The specific objectives of the study are to determine the antibacterial activity of *Azadirachta indica* seed oil, the inhibition rate of *Azadirachta indica* seed oil against *Salmonella typhi, Staphylococcus aureus* and *Pseudomonas aeruginosa* and compare the oil with standard antibiotic against this pathogens.

Materials and Methods

Materials and Methods

Study Area

The study was conducted in Mubi North, seen in figure 1 below. Mubi North lies on latitude 10°32′N to 10°11′N and longitude 13°12′E to 13°35'E, with a total land mass of 506.4Km2 and a population size of 759,045 people [20]

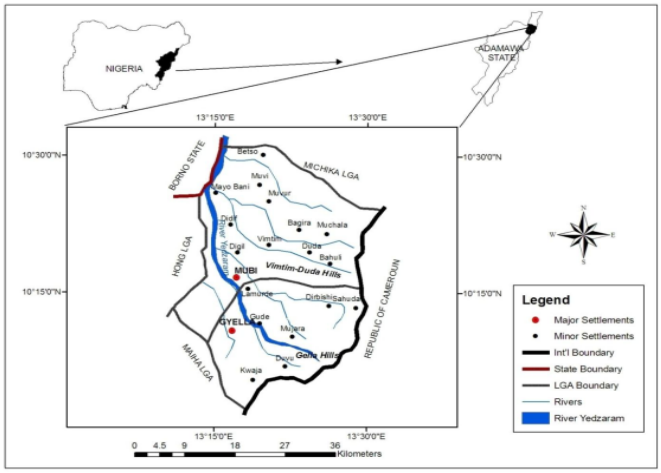

Fig 1 Map of Study Area (Mubi, Adamawa State)

Materials and Methods

Chemical and Reagents

Specialized chemicals/reagents used were of analytical grade and include, Methanol, Sodium carbonate, Sodium Nitrate, Hydrochloric acid, Aluminium Chloride, Sodium Hydroxide, Sodium Phosphate buffer, Hydrogen peroxide and Iron (II) chloride. All chemical and reagents used were analytically graded.

Equipment

Nutrient agar (Titan Biotech), autoclave (pressure stem sterilizer No.25X ALLAMERICAN), nutrient agar plate, incubator (J02714 Electric-Heated Laboratory Incubator), screw capped test tube (leak-tight allowing the tubes were used for transport and storage) were used.

Bacterial Strains

In this study, the bacterial species used were *Salmonella typhi, Staphylococcus aureus* and *Pseudomonas aeruginosa.* The organism were obtained from Microbiology Laboratory section of General Hospital, Mubi.

Traditional Method of *Azadirachta indica* Seed Oil Extraction

The ripe fruits and seeds were collected from *Azadirachta indica* tree, cleaned and washed in ordinary water; and rubbed with palms to remove the skin of the fruits. The cleaned seeds were then spread and dried on the sun to reduce moisture content. This was followed by removing unwanted materials such as stones and dirt by hand picking. Shelling of seeds to obtain clean kernel was carried out by the use of mortar and pestle and winnowing was achieved by holding baskets filled with mixture of seeds and shells at arm length and gradually emptying them. Crushing of kernel to reduce size was carried out by pounding with mortar and pestle. Sieving was carried out using the traditional sieve. Oil extraction was carried out by stirring the fine particles with wooden stick in a bowl and gradually adding warm water to form dough. After the formation of dough, it was pressed with hand in bowl until oil starts gushed out. The oil was then scooped off into an empty dried container.

Confirmation of the Stock Culture

The bacterial strain was subjected to morphological and biochemical confirmation tests. Biochemical tests were performed with Methyl red test, Citrate utilization test, Oxidase test, Catalase test and TSI (Triple Sugar Iron) agar test. All the biochemical tests were performed in specific media (*Salmonella shigella* agar for *Salmonella typhi* and *Pseudomonas aeruginosa;* Mannitol salt agar for *Staphylococcus aureus)* according to the standard methods described in Microbiology Laboratory Manual [21]. The bacterial cultures were grown on nutrient agar plates in the incubator at 37ºC before the process of any biochemical identification test.

Preparation for Reviving the Bacteria

Nutrient agar was prepared for each of the three microorganisms. The media was prepared and autoclaved at 121 °C for 15 minutes. After autoclave the media was poured into the plates. After incubation, the organisms from their previous culture were streaked on nutrient agar plate. The plates were incubated at 37°C for 24 hours. However, in case of *S. typhi* for 48 hours incubation time was followed [21].

Growth on Selective Media

All the organisms from their previous culture were streaked on selective (*Salmonella shigella* agar for *Salmonella typhi* and *Pseudomonas aeruginosa;* Mannitol salt agar for *Staphylococcus aureus)* agar plates for confirmation of the pathogen. The plates were incubated at 37°C for 24 hours. In case of *S. typhi* plates were incubated for 48 hours [22].

Citrate Utilization Test

A small vial was taken into which a slant of the Simmon's citrate agar was prepared and allowed to solidify. A single colony of the bacterium to be tested was touched from a nutrient agar plate by using a needle and carefully streaked onto the slope of the citrate media. The vial was incubated at 37ºC for 24 hours. Over these 24 hours, the organism had the ability to utilize citrate, it changed the colour of the media from deep green to Prussian blue. A negative result keep the colour unchanged [22].

Triple Sugar Iron (TSI)

TSI media was prepared into a screw capped test tube, and was solidified to obtain a slant and butt at the length of the test tube. A single colony of the bacterium to be tested was touched from a nutrient agar plate by using a needle and carefully stabbed at the butt of the TSI (dextrose, lactose & sucrose) follow by slow streaking at the slant. The screw cap of the test tube was loose and the tube was incubated at 37ºC for overnight. After the 24-48 hours of the incubation period, the test tube was examined to observe carbohydrate fermentation, Carbon dioxide (CO_2) and Hydrogen sulfide (H_2S) gas production. The organism was able to ferment all the three sugars, then the butt was turn into yellow colour indicating the production of acid and the subsequent decrease in the pH of the media, whereas a red colour in the slant and butt indicated that the organism being tested was a non-fermenter and the media remains alkaline. Presence of bubbles, splitting and cracking of the medium was the indication of CO_2 gas production. A black precipitation in the butt of the tube was the indication of H_2S production [22].

Catalase Test

A number of autoclaved glass slides were taken, and a drop of the catalase reagent (Hydrogen peroxide) was place on the glass slides. The glass slides were labeled according to the sample being that was tested. A colony for each of the bacteria tested was taken from a nutrient agar plate, and later placed onto the reagent drops on each of the glass slides. An immediate bubble formation indicated a positive result. The same procedure was carried out for clinical and the environmental strains [22].

Oxidase Test

A number of filter papers were taken, and two drops of oxidase reagent (Aminodimethylaniline oxalate) was added onto the filter papers (Whatman, 1MM). The filter papers were labeled according to the sample being tested. A loopful of each bacterium tested (the clinical and environmental strains) was taken from nutrient agar plate and streaked onto the filter paper (Whatman, 1MM). A positive reaction turned the paper from violet to purple within 1 to 30 seconds. Delay reactions were ignored as that might give false positive result [22].

Methyl Red (MR) Test

Six ml (6 ml) of dextrose phosphate (MR-VP) broth was prepared in three test tubes. 3 ml from each of the test tubes were transferred to another three different empty test tubes. All the six test tubes were labeled according to the sample tested and the test that were conducted. All the bacteria samples that were tested were inoculated into 3 ml dextrose phosphate broth (MR-VP broth) contain dextrose and a phosphate buffer and were incubated at 37ºC for 24 hours. After the incubation period was over, five drops of the Methyl red reagent were added into the six test tubes (labeled as MR test) to check the pH of the medium. Development of a red colour indicates a positive result, whereas a yellow colour indicates a negative result [22].

Preparation of Stock Sample

For short-term preservation, 2 ml of Tryptone salt agar (T1N1 agar) butt in a vial was inoculated by stabbing bacterial growth of isolate from nutrient agar plate. Then the vial was kept at 4 ºC for an hour to gelatinize.

After an hour, the surface of the medium was covered with sterile paraffin oil and the vial was stored at room temperature and at 20ºC as well [22].

Methods for Detection of Antibacterial Activity:

Preparation of Bacterial Suspensions

Using a sterile inoculating loop, one or two colonies of the organism tested were taken from the subculture plate. The organism was suspended in 3 ml of physiological saline. The test tube that contains the saline was then vortexed to create an overall smooth suspension [22].

Comparing with the McFarland Solution

McFarland solution was an essential material that was needed before testing the microorganisms for it sensitivity. McFarland standards were used as reference to adjust the turbidity of any given bacterial suspension. This was done to make sure that the number of bacteria was within a given range to standardize the microbial testing. This also helped to avoid any error in result, because if the suspension is too heavy or too diluted, an error might occur for any given anti-microbial agent.

The bacterial suspension prepared was compared with the commercially available McFarland solution 2 (for detection of inhibition rate) and McFarland solution 0.5 (for detection of zone of inhibition by agar disc/well diffusion method). A bacterial suspension was matched with McFarland 2 containing 6×108 colonies per ml. A bacterial suspension was matched with McFarland 2 that contains 1.5×108 colonies per ml [23].

Procedure of Dilution

Bacterial suspension matched with McFarland 2 which was subjected to 10 fold dilution both in saline and in oil. One thousand eight hundred microlitre of oil and saline was taken separately in sets of 7 tubes. Two hundred microlitre of bacterial suspension was added to the 1st tube and 200 µl solution was transferred in the 2nd tube and this procedure was repeated untill 7th tube. Before transferring the solution, every tube was subjected to vortex for uniform mixing. From the 7th tube 200 µl solution was discarded [22].

Detection of Inhibition Rate

One hundred microlitre of the both diluted and undiluted samples were spread on the agar plate containing nutrient agar and from each diluted tube containing saline was spread immediately. One hundred microlitre from each diluted tube containing oil was spread on agar plate after 24 hour of incubation. CFU in saline and CFU in oil of each spread plate was counted and compared. Rate of inhibition in case of every diluted tube was then calculated and averaged to detect actual inhibition rate [22]. Numbers of colonies in countable plates were calculated according to the formula given below;

$$CFU = \frac{Number\ of\ colonies \times reciperocal\ of\ the\ dilution\ factor}{Volume\ of\ plated\ suspensions}$$

Formula of calculation of inhibition Percentage is:

$$\frac{CFU\ in\ saline - CFU\ in\ oil}{CFU\ in\ saline} \times 100$$

Agar Disc and Well Diffusion Method

A filter paper disc/well containing a chemical was placed on the agar, the chemical was diffused from the disc/well into the agar. The solubility of chemical placed on the disc and its molecular size determines the size of the area of chemical infiltration around the disc. Organisms placed on the agar and does not grow in the area around the disc (not susceptible to the chemical). This area of no growth around the disc is known as a "zone of inhibition". Conditions can affect a disc diffusion susceptibility test. When performing these tests certain things were held constant so only the size of the zone of inhibition was variable. Conditions that were constant from test to test include the agar used, the amount of organism used, the concentration of chemical used, and incubation conditions (time, temperature, and atmosphere). The amount of organism used was standardized using McFarland 0.5 standard for this method [22].

Preparation of Dried Filter Paper Discs

Whatman filter paper no. 1 was used to prepare discs approximately 6 mm in diameter, which was placed in a Petri dish after sterilization in autoclave for about 5 minutes [22].

Inoculation on the Nutrient Agar (NA) Plate

A sterile swab was dipped into the bacterial suspension and the tested organisms were suspended in 5 ml of nutrient broth. The swab was rotated against the side of the tube using firm pressure, to remove excess fluid, but the swab was not dripped wet. The dried surface of the Nutrient agar plate was inoculated by streaking the swab three times over the entire agar surface; the plate was rotated approximately 60 degrees each time to ensure an even distribution of the inoculum. The plate was rimmed with the swab to pick up any excess liquid.

Leaving the lid slightly ajar, the plate was allowed to sit at room temperature at least 3 to 5 minutes for the surface of the agar plate to dry before proceeding to the next step [22].

Placement of the Oil Disc and Antibiotic Disc

Oil discs containing 20 µl concentration of *Azadirachta indica* oil was made using filter paper and was then placed on the plates using a sterile forcep. Sterile antibiotic disc was placed on the surface of an agar plate, using a forcep. The forcep was sterilized by immersing the forceps in alcohol. It was then burnt. The disk was gently pressed with the forcep to ensure complete contact with the agar surface. The disk was placed away from the edge of the plates so that it was easily measured. The disks was in place, the plate was inverted, and placed it in a 37 °C incubator for 24 hours [22].

Placement of Oil in the Well

Well was made in agar using a borer. Twenty microlitre of *Azadirachta indica* oil was placed in the well using a pipette. Zone of inhibition was then measured after 24 hours of incubation at 37º C [22].

Measuring Zone Sizes:

Following incubation, the zone sizes were measured precisely using a ruler. All measurements were made, while viewing the back of the petri dish. The zone sizes were recorded on the recording sheet [22].

Data Analysis

Data was analyses using Microsoft Excel [24].

Results

Confirmation of the Clinical Strain

Clinical strain of the three (3) bacterial species *i.e*. *Staphylococcus aureus, Salmonella typhi and Pseudomonas aeruginosa*, obtained from General Hospital Mubi were streaked in the respective selective media in order to determine and confirm the cultural properties of the organisms (Table 1). Selective media are formulated to support the growth of one group of organisms, while inhibits the growth of the other organisms. These media contain antimicrobials, dyes, or alcohol to inhibit the growth of the organisms which are not targeted for study.

Table 1. Cultural Characteristics of the Pathogens on Selective Agar

Organisms	Cultural Characteristics						
	Media	**Size**	**Margin**	**Elevation**	**Form `**	**Pigment**	**Consistency**
Staphylococcus aureus	MSA	Small	Entire	Convex	Circular	Yellow colonies that turn the media Yellow	Smooth
Pseudomonas aeruginosa	Centrimide	Small	Undulate	Raised	Circular	Green colonies that turn the media greenish	Mucoid
Salmonella typhi	XLD	Moderate	Entire	Raised	Convex	Red colonies with black center	Smooth

Biochemical Test Results

The organisms were identified based on following biochemical tests; In the biochemical test result for confirmation of clinical strain, *Staphylococcus aureaus* and *Salmonella typhi* gave a positive result to methyl red reaction test by the development of a red colour in the dextrose phosphate broth when inoculated with the respective bacteria and *Psoudomonas* had a negative result by development of yellow colour.

Staphylococcus aureaus and *Pseudomonas aeruginosa* had the ability to utilize citrate as a colour change of the media is observed from deep green to Prussian blue hence, positive whereas negative result in *Salmonella typhi* where there is no colour change observed in the media. Catalase activity test gave positive result to all the three organisms by immediate bubble formation on the glass slides.

In oxidase activity test, *Staphylococcus aureus*, and *Salmonella typhi* is negative as they did not turn the filter paper from violet to purple within 1 to 30 seconds like *Pseudomonas aeruginosa* that gave positive result.

In triple sugar iron test, *Pseudomonas aeruginosa* gave red colour in the slant and butt, indicating that it is a non-fermenter and the media is alkaline; *Staphylococcus* is a glucose fermenter as the butt turned yellowish in colour, hence acidic for both slant and butt. *Salmonella typhi* had an alkaline slant, and an acidic butt.
There is no bubble observed in all the three organisms. Hence, negative to CO_2 and black precipitation is observed only at the butt of the tube of *Salmonella typhi*. Hence, is positive to H_2S and negative in *Pseudomonas aeruginosa* and *Staphyloccocus aureus* (Table 2).

Table 2. Biochemical Characterization of the Pathogens

Organisms	Biochemical Tests							
	Methyl Red Reaction Test	Citrate Utilization Test	Catalase Activity Test	Oxidase Activity Test	Triple Sugar Iron			
					Slant	Butt	CO_2	H_2S
Staphylococcus aureus	+	+	+	_	Acidic	Acidic	_	_
Pseudomonas aeruginosa	_	+	+	+	Alkaline	Alkaline	_	_
Salmonella typhi	+	_	+	_	Alkaline	Acidic	_	+

Comparison of the Growth of Organisms in Saline and Oils
After incubation fewer colonies appeared on the agar plate containing *Azadirachta indica* seed oil than saline which indicates that this oil had antimicrobial activity against the selected pathogens. Number of colonies of the matted plate and those plates which had more than 300 colonies were determined by back calculation.
Determination of inhibition Percentage
The number of colonies found in saline and *Azadirachta indica* seed oil showed that each set were then averaged to determine inhibition rates.
Total Viable Count of *Staphylococcus aureus* in Saline and in Oil
There is no colony found from 10^{-4} to 10^{-7} dilution of *Azadirachta indica* seed oil whereas there were observed colonies in saline and the Colony Forming Unit (cfu) from 10^{-1} to 10^{-3} is minute or minimal compared to dilution in saline. Hence, giving 100% inhibition of pathogens from 10^{-4} to 10^{-7} and 99.99% from 10^{-1} to 10^{-3} dilution in oil (Table 3).

Table 3. Total Viable Count of *Staphylococcus aureus* in Saline and in Oil

Organism	Dilution of the bacterial suspensions with oil and saline	Saline CFU/100 µl	*Azadirachta indica* seed Oil	
			CFU/100 µl	% of inhibition
Staphylococcus aureus	10^{-1}	6.6×10^{6}	825	99.99
	10^{-2}	6.6×10^{5}	43	99.99
	10^{-3}	6.6×10^{4}	8	99.99
	10^{-4}	6.6×10^{3}	0	100
	10^{-5}	6.6×10^{2}	0	100
	10^{-6}	6.6×10^{1}	0	100
	10^{-7}	6	0	100

Total Viable Count of *Pseudomonas aeruginosa* in Saline and in Oil

There is no colony found from 10^{-3} to 10^{-7} dilution of *Azadirachta indica* seed oil whereas there were observed colonies in saline and the Colony Forming Unit (cfu) at 10^{-1} and 10^{-2} is minute or minimal compared to dilution in saline. Hence, giving 100% inhibition of pathogens from 10^{-3} to 10^{-7} and 99.99% from 10^{-1} to 10^{-2} dilution in oil (Table 4).

Table 4. Total Viable Count of *Pseudomonas aeruginosa in* Saline and in Oil

Organism	Dilution of the bacterial suspensions with oil and saline	Saline CFU/100 µl	*Azadirachta indica* seed Oil CFU/100 µl	% of inhibition
Pseudomonas aeruginosa	10^{-1}	6.7×10^{6}	577	99.99
	10^{-2}	6.7×10^{5}	94	99.99
	10^{-3}	6.7×10^{4}	0	100
	10^{-4}	6.7×10^{3}	0	100
	10^{-5}	6.7×10^{2}	0	100
	10^{-6}	6.7×10^{1}	0	100
	10^{-7}	6	0	100

Total Viable Count of *Salmonella typhi* in Saline and in Oil

There is no colony found at 10^{-6} and 10^{-7} dilution of *Azadirachta indica* seed oil whereas there were observed colonies in saline and the Colony Forming Unit (cfu) from 10^{-1} to 10^{-5} is minute compared to dilution in saline. Hence, giving 100% inhibition of pathogens at 10^{-6} and 10^{-7}, 99.72% inhibition of pathogens from 10^{-1} to 10^{-3}, 99.17% at 10^{-4} and 99.68% at 10^{-5} dilution in oil (Table 5).

Table 5. Total Viable Count of *Salmonella typhi* in Saline and in Oil

Organism	Dilution of the bacterial suspensions with oil and saline	Saline CFU/100 µl	*Azadirachta indica* seed Oil CFU/100 µl	% of inhibition
Pseudomonas aeruginosa	10^{-1}	6.3×10^{6}	1.79×10^{4}	99.72
	10^{-2}	6.3×10^{5}	1.75×10^{3}	99.72
	10^{-3}	6.3×10^{4}	171	99.72
	10^{-4}	6.3×10^{3}	52	99.17
	10^{-5}	6.3×10^{2}	2	99.68
	10^{-6}	6.3×10^{1}	0	100
	10^{-7}	6	0	100

Inhibitaion of Growth of Various Organisms by Neem Oil

Table 6 shows that *Staphylococcus aureus* and *Pseudomonas aeniginosa* have 99.99% and *Salmonella typhi* having the least average percentage of inhibition rate in *Azadirachta indica* seed oil has 99.72%.

Table 6. Inhibition of Growth of various Organisms by Neem Oil

Organisms	Averaged Percentage of Inhibition *Azadirachta indica* seed Oil
Staphylococcus aureus	99.99%
Pseudomonas aeniginosa	99.99%
Salmonella typhi	99.72%

Selective Antimicrobial Activity Test by Means of Antibiogram Method

The results of antibacterial activity of *Azadirachta indica* oil screened against the bacterial pathogens using agar well diffusion and the zone of inhibition is measured in mm diameter. The area of inhibition where the growth of microorganisms was inhibited by *Azadirachta indica* seed oil was observed to be significant with

all the three pathogens used when compared with the control (Table 7). All the three (3) bacterial strains were subjected to the standard disc diffusion test with a control antibiotic and paper disc and well containing oil. Control antibiotic for S*taphylococcus aureus, Salmonella typhi* was Chloramphenicol and for *Pseudomonas aeruginosa* was Cefepime. The zone diameter of inhibition interprets the resistance and sensitivity of the organisms to the respective antibiotic and oil. Presence of zone of inhibition around oil disc or well containing oil means that this oil has antibacterial activity against the selected pathogens.

Table 7. Zone of Inhibition in Response to Oil and Antibiotic

Organisms	**Zone of Inhibition (mm)**			
	Antibiotic	**Paraffin Oil**	***Azadirachta indica* seed Oil**	
			Well	**Disc**
Staphylococcus aureus	26	0	15	4
Pseudomonas aeniginosa	29	0	18	6
Salmonella typhi	22	0	10	5

Discussion

Medicinal plants and their derivatives have found several applications over the years as traditional therapies, food preservatives and pharmaceuticals [25]. This is because they harbor some bioactive compounds which affords them the varying potentials [11]. This study explored the antibiotic possibilities of seed oil *Azadirachta indica* a tropical plant. In this investigation, the percentage inhibition of *Azadirachta indica* seed oil detected against the study bacterial stereotypes was over 99% which corresponds with the findings of Zhan *et al.,* in their *in vitro* studies [26]. Tuhin *et al.,* determined *Azadirachta indica* seed oil inhibitory effect against *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhi* and *Staphylococcus aureus*, through broth dilution method and Agar disc diffusion methods.The respective minimum inhibitory concentration obtained against *Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* were 1:32, 1:32, 1:8 and 1:16. A 19 mm zone of inhibition was generated by *Azadirachta indica* seed oil against *Staphylococcus aureus*, while cefepime used as standard displayed a diameter of 30 mm against similar microbe. Likewise, a notable zone of inhibition of the seed oil against *Escherichia Coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* were observed [27].

Similar trend of antibacterial effects (Table 7) showed that the zone of inhibition on all study pathogens were significant ranging from 22 to 29 mm detected through Agar disc diffusion assay. The presence of bioactive components such as nimbin, azadirachtin, salin picrin [28] and others [11] identified in *Azadirachta indica* seed oil may serve as the bases of the oil's pharmacological effects as previous literatures have established strong relationship between plants bioactive components and a number of therapeutic effects [7,8,9]. The hydrophobicity of plants essential oils have also been reported to tamper with the semipermeable nature of bacterial membrane, thereby, altering the cell structure into a more permeable nature. As a result, critical molecules leak out of the cell, therefore, causing cell death [29]. This may also serve as additional antibiotic mechanism displayed by *Azadirachta indica* seed oil.

Conclusion

From this study, it can be concluded that *Azadirachta indica* seed oil possess antibacterial potentials having exerted superb inhibitory effects against both gram negative and positive bacteria. This investigation alongside with previous studies provides support to the antibacterial properties of this oil. It is therefore, taken into account as one of the most promising of all plants and hence should eventually benefit one and all on this planet. Additional *in vivo* studies and clinical trials would be needed to justify and further evaluate the potential of this oil as an antibacterial agent in topical or oral applications.

Studies are also required to confirm this antibacterial activity of *Azadirachta indica* seed oil and to separate the active constituents and evaluate their antibacterial activity and to identify exact antimicrobial compounds from the oil. The compounds need to be identified and purified using high performance liquid chromatography (HPLC) or other high throughput technique.

Data Availability Statement
The authors confirms that the literatures supporting this study are cited and acknowledged in the reference list.
Conflict of interest
The authors declares no conflict of interest.
Ethical standards
The study conform with ethical standards.
Availability of data and material
The datasets generated and are available with the corresponding author.
Authors' contributions
AN: Conceptualization, methodology, investigation, data collection, Formal analysis, and Writing.
IY: Conceptualization, methodology, critical review and editing of the manuscript.

References

1. Deckard, A. (2015). 11 Powerful Antibacterial Essential Oils. 90-99. https://healthyfocus.org/8-powerful-antibacterial-essential-oils/ Accessed August 2, 2026.
2. Rodreguez, T. (2015). Essential Oils Might Be the New Antibiotics. Today and tomorrow's Printers and Publishers, New Delhi, India. https://www.3rdactmagazine.com/wellness/health-wellness/essential-oils-might-be-the-new-antibiotics-6/ Accessed August 2, 2026.
3. Gayathri, R. M., Vishnupriya, V., R.Gayathri, R. and Geetha, R. V. (2016). Anti-Bacterial Activity of Neem Oil on Oral Pathogens – An In vitro Study. International Journal of Pharmaceutical Science Review Research, 39(1): 219-220. https://globalresearchonline.net/journalcontents/v39-1/42.pdf Accessed August 2, 2026.
4. Ahmad, A., Husain, A., Mujeeb, A., Khan, S. A., Najmi, A. K., Siddique, N. A., Damanhouri, Z. A., and Firoz, A. F. (2013). A review on therapeutic potential of Nigella sativa: A miracle herb. Asian Pacific Journal of Tropical Biomedicine, 3 (5): 337–352. https://doi.org/10.1016/S2221-1691(13)60075-1
5. Kumar, V. S. and Navaratnam, V. (2013). Neem (Azadirachta indica): Prehistory to contemporary medicinal uses to humankind. Asian Pacific Journal of Tropical Biomedicine, 3(7), 505–514. https://doi.org/10.1016/S2221-1691(13)60105-7
6. Kumar, V. S. and Navaratnam, V. (2013). Neem (Azadirachta indica): Prehistory to contemporary medicinal uses to humankind. Asian Pacific Journal of Tropical Biomedicine, 3(7), 505–514. https://doi.org/10.1016/S2221-1691(13)60105-7
7. Sartoratto, A., Ana, L.M.M., Delarmelina, C., Figueira, G.M., Marta, C.T.D., Rehder, V. L. G. (2004). Composition and Antimicrobial Activity of Essential Oils from Aromatic Plants Used in Brazil. Brazilian Journal of Microbiology, 35 (4): 275-280. https://doi.org/10.1590/S1517-83822004000300001
8. Mosaddek, A. S. M. and Rashid, M. M. U. A. (2008). Comparative study of the anti-inflammatory effect of aqueous extract of neem leaf and dexamethasone. Bangladish Journal Pharmacology. 3: 44-47. https://doi.org/10.3329/bjp.v3i1.836
9. Patil, P. R., Patil, S. P., Mane, A. and Verma, S. (2013). Antidiabetic activity of alcoholic extract of Neem (Azadirachta indica) root bark. National Journal of Physiology Pharmaceutical Pharmacology, 3: 142-146. https://doi.org/10.5455/NJPPP.2013.3.134-138
10. Hulin, V., Mathot, A. G., Mafart, P. and Dufosse, L. (1998). Les proprietes anti-microbiennes des huiles essentielles et composes daromes. Sciences des Aliments, 18: 563-582. https://agris.fao.org/search/en/records/64722d6f53aa8c896301987a Accessed August 2, 2026.
11. Ganguli, S. 2002. Neem: A therapeutic for all seasons. Current Science, 82: 1304. https://www.currentscience.ac.in/ Accessed August 2, 2026.
12. Okpanyi, S. N. and Ezeukwk, G. C. (1981). Anti-inflammatory and anti-pyretic activities of Azadirachta indica. Plantation Medication, 41: 34-49. https://doi.org/10.1055/s-2007-971670
13. Khattak, S. G., Gilani, S. N. and Ikram, M. (1985). Antipyretic studies on some indigenous Pakistani Medicinal Plants. Journal Ethnological Pharmacology, 14: 45-51. https://doi.org/10.1016/0378-8741(85)90027-3
14. Fujiwara, T., Takeka, T.O., Gihara, Y., Shimizu, M., Nomura, T. and Tomuta, Y. (1982). Studies on the structure of polysaccharides from the bark of Medica Azardichta. Chemistry Pharmaceutical Bulletin, 30: 4025-4030. https://doi.org/10.1248/cpb.30.4025
15. Pillai, N. R. and Santhakumari, G. (1984). Hypoglycemic activity of Melia azadirachta. Indian Journal of Medicinal Research. 74: 931-933. https://www.ncbi.nlm.nih.gov/pmc/?term=Pillai+Santhakumari+Hypoglycemic+activity+of+Melia+azadirachta Accessed August 2, 2026.
16. Sinha, K. C., Riar, S. S., Tiwary, R. S., Dhawan, A. K., Bhadhan, J., Thomas, P., Kain, A. K. and Jain, R. K. (1984). Neem oil as a vaginal contraceptive. Indian Journal of Medicinal Research, 79: 131-136. https://pubmed.ncbi.nlm.nih.gov/6724648/ Accessed August 2, 2026.
17. Alzohairy, M. A. (2016). Therapeutics role of Azadirachta indica (Neem) and their active constituents in diseases prevention and treatment. Evidence-Based Complementary and Alternative Medicine, 2016, Article 7382506. https://doi.org/10.1155/2016/7382506
18. Sharma, S., Gupta, P., & Kumar, N. (2021). Phytochemical profiling and antimicrobial activity of Azadirachta indica seed oil against human pathogens. Journal of Applied Natural Science, 13(2), 596–604. https://doi.org/10.31018/jans.v13i2.2671
19. Bandyopadhyay, U., Biswas, K., Sengupta, A. (2004). Clinical studies on the effect of neem (Azadirachta indica) bark extract on gastric secretion and gastroduodenal ulcer. Life Science, 75: 2867-2878. https://doi.org/10.1016/j.lfs.2004.04.050
20. Ray, H. H. (2007). "The effects of physical techniques on soil conservation in Mubi and environs, Adamawa State, Nigeria", Journal of Sustainable Development in Agriculture and Environment, Vol. 3 pp. 112-121. https://www.academia.edu/144724815/Society_for_Community_and_Communication_Development_Research_of_the_4_th_Biennial_Conference_of_the_Society_for_Community_and_Communication_Development_Research_SCCDR_Held_at_the Accessed August 2, 2026.

21. Cappuccino, J.G. and Natalie, S. (2013). Microbiology: A laboratory manual. Peason new international edition: 13-182. https://www.amazon.com/Microbiology-Laboratory-James-G-Cappuccino/dp/0321840224 Accessed August 2, 2026.
22. Nishat, A. S. (2017). Comparative study on the antibacterial activities of Neem oil, Mustard oil and Black seed oil against Staphylococcus aureus, Klebsiella pneumoniae, Bacillus cereus, Salmonella typhi and Pseudomonas aeruginosa. A Dissertation Submitted to Brac University, Department of Mathematics and Natural Sciences BRAC University Dhaka, Bangladesh. https://dspace.bracu.ac.bd/xmlui/handle/10361/8507 Accessed August 2, 2026.
23. McFarland, J. (1907) Nephelometer: An Instrument for Estimating the Number of Bacteria in Suspensions Used for Calculating the Opsonic Index and for Vaccines. Journal of the American Medical Association, 14, 1176-1178. https://doi.org/10.1001/jama.1907.25320140022001f
24. MSO (2010). Microsoft Office: Application Package (Excel). https://www.microsoft.com/en-us/microsoft-365/microsoft-office Accessed August 2, 2026.
25. Burt, S. A. (2004). Essential oils: their antibacterial properties and potential applications in foods: a review; Internatinal Journal of Food Microbiology, 94 (3): 223-253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022
26. Zahn, R., Moll, J., Garrido, G., Krueger, F., Huey, E. D. and Grafman, J. (2007). Social concepts are represented in the superior anterior temporal cortex. Proc. Natl. Acad. Sci. USAg; 104: 6430–6435. https://doi.org/10.1073/pnas.0607061104
27. Tuhin, J., Zinnat, A. B. and Sayeeda, S. (2007). Effect of neem oil on some pathogenic bacteria. Bangladesh Journal of Pharmacology; 2: 71-72. https://doi.org/10.3329/bjp.v2i2.573
28. Mishra, A. and Dave, N. (2013). Neem oil poisoning: Case report of an adult with toxic encephalopathy. Indian Journal of Critical Care Medicines, 17 (5): 321–322. https://doi.org/10.4103/0972-5229.120330
29. Knobloch, K., Weigand, H., Weis, N., Schwarm, H.M., Vigenschow, H. (1986). Action of terpenoids on energy metabolism. Progress in Essential Oil Research: 16th International Symposium on Essential Oils, BMC complementary and alternative medicine, 13 (130): 429-445. https://doi.org/10.1515/9783110855449-039