

PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF NEEM SEED OIL (*AZADIRACHTA INDICA*) ON BACTERIA ISOLATES

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ABSTRACT: Antibacterial activity of neem (*Azadirachta indica*) seed oil extract was investigated using microbial growth inhibition zone. The neem seed oil was obtained by the cold extraction method using ethanol as an organic solvent of which 42ml of oil was obtained from 70g of neem seeds, with 40g (57%) of residue. Oil extracted was screened for its antibacterial properties and phytochemical components. The test organisms used were *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* had the highest zones of inhibition while *Escherichia coli* had the least zone of inhibition. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The Phytochemical screening of the sample revealed the presence of Tannin, Alkaloid and Hydrogen cyanide.

KEY WORDS: antibacterial, neem, inhibition, phytochemical, extract.

INTRODUCTION

The emergence of drug resistant bacteria is a matter of concern (Ncube *et al.*, 2008) as it is a serious global problem, as well as clinicians and the pharmaceutical industry (De *et al.*, 2002). Use of herbal medicines in the developed world continue to rise because they are rich source of novel drugs and their bioactive principles form the basis in medicine, pharmaceutical intermediates and important compounds in synthetic drugs (De *et al.*, 2002 and Ncube *et al.*, 2008). The global scenario is now changing towards the use of non-toxic plant products having medicinal values (Biswas *et al.*, 2002). Screening medicinal plants for biologically active compounds offer clues to developing newer antimicrobial agents.

Neem (*Azadirachta indica*), the versatile medicinal plant is the source of several compounds having diverse chemical structure and biological effects (Biswas *et al.*, 2002). A significant amount of research has already been carried out during the past to understand the chemistry and medicinal uses of different parts of neem for use therapeutically and industrially (Maragathavalli *et al.*, 2012). *Azadirachta Indica* is now used in traditional medicine as a source of many therapeutic agents. *A. indica* (seed) is known to contain antibacterial, antifungal activities against different pathogenic microorganisms and antiviral activity against vaccinia, chikungunya, measles and coxsackie B Viruses (Biswas *et al.*, 2002). Neem seed have been shown to exhibit wide pharmacological activities including; antioxidant, antimalarial,

antimutagenic, anti carcinogenic, anti- inflammatory (Maragathavalli *et al.*, 2012). The biological activities are attributed to the presence of many bioactive compounds in different parts of the plant.

Azadirachta indica is used in India and Africa to make all sort of consumer products, such as pesticides and insect repellents, soaps, cosmetics, toothpaste, antiseptic, gargle, ointment, poultices, lubricants, fertilizers, fuel for oil lamps, rope, glue and tannin from bark fibre etc. (Verge, 2001). It is important to mention that because neem products are used for human consumption and medication, exposure to neem in the process of treating plants with neem oil poses no threat to humans or other animals. Moreover, neem is not harmful to beneficial insects, affecting only those insects feeding on plants treated with neem (Khanna, 2001). Since most predator insect does not also feed on plants, they are not harmed by the presence of neem. This implies that there is product safety. Neem (*Azadirachta indica*) is also environmental friendly in the sense that it biodegrade in a matter of weeks when exposed to sunlight or in soil.

Furthermore, apart from the above mentioned uses of neem (*Azadirachta indica*) seed oil, numerous tests have shown neem oil to be effective as an insecticide, miticide, fungicide, nematocides and as an insect antifeedents and repellents (Khanna, 2001). More than 135 compounds have been isolated from neem. The compounds have been divided into 2 major groups- isoprenoids and others (Biswas *et al.*, 2002). The isoprenoids include diterpenoids, azadirone, gedunin, nimbin, salanin and azadirachtin. The non-isoprenoids include proteins, carbohydrates, sulphurous compounds, polyphenols, such as flavonoids and aliphatic compounds. Researchers have detected several medicinal effects of neem including antidiabetic effect, antifertility effect, antitumour effect, antiulcer effect, antimalarial effect and antipyretic effect (Shukla *et al.*, 2003; Singh *et al.*, 2007). Previous studies have shown that neem has antibacterial activity (Vanka *et al.*, 2001; Rao *et al.*, 2006).

Previous studies have shown the effect of acetone extract of neem (*Azadirachta indica*) seed to suppress the growth of several species of some pathogenic bacteria

Objectives of this study is to determine the phytochemical screening of the extract of neem (*Azadirachta indica*) seed oil, as well as determine the antibacterial effect of extract of neem seed oil on four bacterial isolates.

The fact that many traditional remedies are of therapeutic value is no longer in doubt. Some of the important drugs which have revolutionized modern medical practice have almost all been employed in traditional medicine (Ncube *et al.*, 2008). These include curative alkaloids, reserpine, penicillin and cortisone (Isoun, 2001). Isoun (2001) also reported that synthetic chemicals are usually toxic, non-renewable, and costly and their production often leads to environmental pollution. On the other hand, natural products especially those with those with long history of use in traditional medicine are renewable resources for pharmaceuticals raw materials (Pillia *et al.*, 2004). They are usually locally available and their cultivation, harvest and processing are less costly and are environmentally friendly (Biswas *et al.*, 2002).

Researchers are thus, returning to natural sources of biologically active chemicals gotten from plants, insects, marine invertebrates, fungi and bacteria (Pillia *et al.*, 2004). About a decade ago some 50,000 Neem trees were planted over 10km² on the plains of Arafat to provide shade for Muslim pilgrims during hajj. (Khanna, 2001). The neem trees plantation had led to a marked impact on the areas microclimate, microflora, microfauna, and sandy soil properties and when full grown, could provide shade to 2 million pilgrims. It is equally ancient belief that, neem growing inside in the house can keep the surrounding air clean of impurity and there by control environmental pollution (Khanna, 2001).

The neem tree also, renders the same uses to Nigerians in the north (Sara and Folorunsho, 2001). The neem trees (*Azadirachta indica*), stands out for versatility. It is variously used for pesticides, medicinal antiseptics, in desert control (Aggrawal *et al.*, 2005), as well as for fuel wood production. The tree is nicknamed “The Village Pharmacy” and also “An Ancient Cure for Modern World” in India (Aggrawal *et al.*, 2005). Neem trees play a very active role in the afforestation project in the northern part of the country. It is perfectly adapted to the arid and semi-arid ecologies (Isoun, 2001). The tree has fully established in every part of Nigeria, despite been native to East India, and Burma. It occupies a pride of place in programmes of the Centre for arid zone studies (Sara and Folorunsho, 2001).

According to Hassan (2001), the uses of the tree are many, these include fire wood, charcoal, timber, poles, furniture, medicine (leaves, bark, roots), fodder (foliage, oil seed cake), bee-forage, shade, soil fertility, soil conservation and erosion control, wind breaks, ornamental and insecticides (*Azadirachta*). The wood is mainly used as fuel or house construction and fencing. In many countries, it is therefore preferred for construction of carts, agricultural tools and handles (Hassan, 2001). Furthermore, (Vierge, 2001) reported that the leaves with a protein content of 15% could be used as fodder for camels, sheep and goats. At low fiber content, they have nutritional value comparable with leguminous leaves. The oil cake is a good fertilizer and an effective insecticide so that after fertilizing of agricultural crops, attack by termites and diverse insects is effectively reduced (Vierge, 2001).

The effect as an insecticide is attributed to the content of azadirachtin found in seeds and leaves and which even at low concentrations impede feeding and moulting of larvae and caterpillars. The living tree also repels insects and is less susceptible to attack of parasites than many other species and it has not been known to be toxic to man (Hassan, 2001). In ancient India, almost all parts of neem trees are found medicinally effective. Bark, leaves, fruit, oil and sap help to cure various skin diseases, venereal diseases (syphilis), tuberculosis, etc. They are also used as an antidote (scorpion or snake bites) antiseptic, astringent anthelmintic, diuretic, tonic, antiperiodic and remedy against rheumatism and sprains (Hassan, 2001).

The neem seed oil (NSO) is obtained from the seed kernels of the neem tree and the process of obtaining it is a fairly simple process. Crushing the kernels and extracting them with water is the most effective way for village people to obtain neem insecticides (Khanna, 2001). According to him scientists have developed more advanced processes and means to convert neem extracts to form granules, dust, wettable powders or emulsifiable concentrations.

Scientists have also developed Neem ingredients, to increase shelf life of products or to reduce photo-toxicity, the damage to sensitive plants (Khanna, 2001). Neem oil has been reported to be clean burning (Khanna, 2001). The oil is 50% of kernel's weight. Neem Seed Oil (NSO) is very bitter with a garlic, sulphur smell (Khanna, 2001). It contains Vitamin B and other essential acids (Khanna, 2001). The oil is found to have the following fatty acids, oleic acid, stearic acid, palmitic acid, linoleic acid, and various lower fatty acids, (Khanna, 2001). The percentages vary from sample to sample depending on the place and time of collection of the seeds (Khanna, 2001). Khanna also reported that the compounds isolated from the kernels have shown diverse effects including repellent, feeding and oviposition deterrent, growth regulating, sterilant and impairing hatching of eggs. No plant or chemical is known to have such effects on insects. Neem oil is very effective on plants disease like rust and powdery mildew (Khanna, 2001).

THE ANTIMICROBIAL ACTIVITIES OF THE NEEM SEED OIL

Akinniyi (2001), in an overview of uses of neem plants for medicinal, cosmetic and other purposes enumerated the various antimicrobial effects of the neem Seed oil ranging from its antifungal, antiviral to antibacterial properties. New neem extract have been effective against certain fungi which infect the human body (Khan and Wassilow, 2007).

Researchers show results that neem preparations were toxic to cultures of fourteen common fungi belonging to the following genera. Trichophyton, an "athlete's foot" fungus that infects hair, skin, and nails. Epidermophyton – a ringworm that invades both skin but rarely the nails; Microsporum – a ringworm that invade hair and skin but rarely the nails Trichosporum – a fungus of the intestinal tract, Geotrichum – a yeast like fungus which causes infection of the bronchi, lung and numerous membranes; Candida – a yeast like fungus which is part of the normal mucous flora, but can get out of control and lead to lesions of mouth, vagina, skin, hands and lungs (Khan and Wassilow, 2007). Neem oil has been reported to be capable of suppressing several species of pathogenic bacteria (Patel, 2002; Schneider *et al.*, 2006). The flower has also been reported to have antimicrobial activities (Akinniyi, 2005).

JUSTIFICATION OF THE RESEARCH

Nigeria is the most populous black nation of the world. The harsh economic situation of the nation has increase the poverty rate and has cut off a lot of the populace from affording decent and orthodox medicines. In such rural settings the use of herb and native medicines has been the other of the day. Neem plant abound both in the north, south, west and eastern part of the nation. The leaves are used mainly for the treatment of malaria but the seeds ripen and fall off and are very abundant yet unused by the locals. To this end we decided to investigate the content and medicinal value of the seed oil

MATERIALS AND METHOD

The seeds were collected from neem tree from Federal University Of Technology and then depulped, washed and air dried Ahmed and Stroll (2006). The extraction of the neem seed oil was done using the cold extraction method according to Vietmeyer (2002). 70g of the Neem seeds was pounded with mortar

and pestle and dissolved in 300ml of analytical grade of organic solvent- ethanol in a volumetric flask. The mixture was stirred occasionally with a glass rod. After 2 hours, the mixture was filtered through Whatman No 1 filter paper. The filtrates were then evaporated using rotary evaporator.

The tests organisms used were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi*. These are common pathogenic organisms in our environment. The test organisms were gotten from stock cultures obtained from Federal Medical Centre (FMC) Owerri, Imo state. They were sub cultured in nutrient broth, incubated for 24hr.

PREPARATION OF MEDIA

Preparation of Nutrient Agar was prepared aseptically in the laboratory according to manufacturer's specifications inscribed on the labels of the media. 28g of dehydrate Nutrient agar base medium was dissolved in about 800ml of distilled water. The mixture was heated in a water bath until the agar melted. It was made up to 1litre and its pH checked to conform to standard 7.2 – 7.6, (Adegoke *et al.*, 2009). The prepared medium was used for plate preparation all through this study

IDENTIFICATION OF ISOLATES

GRAM STAINING TECHNIQUES/ MICROSCOPY

A smear of bacteria was prepared on a clean, grease- free slide; allow air-drying and then heat fixed. The fixed smear was flooded with crystal violet stain for 30-60 seconds. The dye was drained off and Lugol's iodine applied and left for 60 seconds. This was drained and the slide washed under the tap. Ethanol was then used to decolorize the smear and washed off after about 10 seconds. The slide was flooded with Safranin for 60 seconds. This was later drained and the slide washed and blot dried. The preparation was then examined under the microscope using the oil immersion lens. This reaction was used to separate bacteria into two main groups: the Gram-positive (which give a blue to purple reaction to stain) and Gram-negative (pink to red reactions to stain) according to (Junaid *et al.* , 2006).

BIOCHEMICAL TESTS

According to (Jawatz *et al.*, 2005) All test strains were re-isolated on nutrient agar (Oxide) to obtain pure culture and identification was confirmed by standard bacteriological methods as listed below:

OXIDASE TEST

The test identifies cytochrome C oxidases, an enzyme found in obligate aerobic bacteria. The enzyme if present will catalyse the transport of electrons between the electron donors in the bacteria and a redox dye, N1 N-Dimethylphenylenediamine Dihydrochloride. The dye was reduced to purple blue colour. 1% aqueous solution of the oxidase reagent was prepared by dissolving 1g of tetramethyl-p-phenylenediamine Dihydrochloride. This was used immediately. Small piece of whatman filter paper was soaked in the freshly prepared solution. With the aid of a glass rod, a small amount of the bacterial

colony was rubbed onto the filter paper. A purple-blue colour within 10 seconds showed a positive result (Jawetz, *et al.*, 2005).

CATALASE TEST

This identifies catalase, an enzyme found in obligate aerobes and in most facultative anaerobes which catalyse the breakdown of hydrogen peroxide into water and free oxygen. This was done using the method described by Fawole and Oso (2005) 3% of H₂O₂ was prepared by adding 3mls of H₂O₂ to 97ml of distilled water. Some drops of the 3% H₂O₂ was mixed with a loop full of the colonies on a clean microscope slide. An immediate bubbling (effervescence) shows a positive reaction.

INDOLE TEST

The indole test for *Escherichia coli* was done using Kovac's reagents, para-Dimethylaminobenzaldehyde. This reagent was prepared as follows: this was done as follows using the method described by Fawole and Oso (2005). 5g of the Kovac's reagents was mixed with 75ml of amyl alcohol, then heat applied to a temperature of 50-55⁰ C, then 25ml of hydrogen chloride was added. When the organism is inoculated into a test tube, with the reagent added, the presence of pink colouration in a ring form will indicate positive results.

COAGULASE TEST

This test is used for the identification of *Staphylococcus species* and also to differentiate *S. aureus* from *S. epidermidis* and *S. saprophyticus*. A drop of physiological saline was placed on each end of a slide, and emulsions of the test organisms prepared. A loop full of plasma was added to one of the emulsions and mixed gently. A positive result shows clumping of that organism within 10 seconds. The second emulsion served as a control. *S. aureus* is coagulase-positive (i.e. produces coagulase) while *S. saprophyticus*, and *S. epidermidis* are coagulase negative (Jawetz *et al.*, 2005).

METHYL RED TEST:

The methyl red test was employed to detect the production of sufficient acid during fermentation of glucose. The test organism was inoculated into glucose-phosphate-peptone water medium and was incubated at 37⁰C for 48 hours. At the end of the incubation, 2-3 drops of 0.02% methyl red reagent was added to 5ml of the culture and observed for any colour change (Schumann *et al.*, 2002).

SUGAR UTILIZATION TEST

This test was carried out to determine the ability of the isolates to ferment different sugars to form acids accompanied by the evolution of gas, usually CO₂. One percent peptone water containing 1% of the appropriate sugar (lactose, sucrose, glucose), bromocresol purple indicator were added to the test tube. Durham's tubes were placed inside each of the tubes containing the liquid medium and sterilized by

autoclaving. After cooling, the sterile medium was inoculated with a loopful of the test bacterial culture incubated at 37°C for 48hours. The medium was examined for a colour change from purple to yellow, which indicated acid production; gas production in the tubes was also noted. (Junaid *et al.*, 2006).

TRIPLE SUGAR IRON AGAR (TSIA)

This test is used to determine the ability of certain bacteria to utilize a specific sugar incorporated into a basal growth medium with or without the production of gas along with a possible hydrogen sulphide (H₂S) production. TSI agar was prepared in slants; the medium was inoculated by stabbing the butt and streaking the slant with a loopful of the appropriate isolate, incubated at 37°C for 48hours. The production of gas is marked by cracks in the agar as well as air gap at the bottom of the test tube while H₂S production is indicated by the presence of black precipitates which indicates the reduction of sodium thiosulphate to hydrogen sulphide. This was done using the method ascribed by Hewitt and Vincent (2009)

VOGUES PROSKAUER TEST (BARRIT TEST)

This tests the ability of some bacteria to ferment carbohydrates with the production of acetyl methyl carbinol or its reduction product 2, 3-butylene glycol in the medium. A loopful of the test organism was inoculated into a test tube which contained 10ml of glucose-phosphates-peptone water and incubated at 37°C for 48hours. At the end of the incubation period, 3 drops of 4% potassium hydroxide and 6 drops of 5% of alpha-naphthol in absolute alcohol was added to 5ml of the 48hours culture and observed for any colour change (Junaid *et al.*, 2006).

PREPARATION OF STANDARD

This was done aseptically to determine the sensitivity or resistance of the isolates to Ampicillin. 250mg of Ampicillin capsule was dissolved into 5ml of sterilized test tubes containing distilled water then mixed together (Junaid *et al.*, 2006).

ANTIMICROBIAL SCREENING

The modified agar well diffusion method was employed to determine the antimicrobial activities for the ethanolic Neem seed oil extracts. Different concentrations of extracts, 100%, 50% and 33.3% were prepared; this is to know the least of the level of concentration that will be required for the culture to grow (Collins *et al.*, 2005). About 0.1ml of the standardized 24hour old culture of the tested organisms in Nutrient broth was spread unto sterile prepared Nutrient agar plates and allowed to set. With the aid of a sterile cork borer, hole of 6mm in diameter were bored on the plates, about 0.5ml of each concentrations of the extracts was dispensed into the hole and then allowed to stand for about 15minutes (Collins *et al.*, 2005). Also 0.1ml of the prepared ampicillin solution (standard) was dispensed into the

hole. These were then incubated at 37°C for 24 hours. At the end of the period, the zones of inhibition were measured using scientific ruler and the diameters were recorded (Junaid *et al.*, 2006).

The Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) was determined by measuring about 5ml of nutrient broth into empty sterilized tubes. 1ml of the different concentration was then added. This was then incubated for 24 hours at 37°C. The tube with the least concentration of extract that showed least growth (-ve) was determined as the MIC while the negative tubes were pour plated on Nutrient Agar and incubated for 24 hours at 37°C. The tube with the least concentration of the extract that showed no growth at the concentration was reported as the MBC (Junaid *et al.*, 2006).

PHYTOCHEMICAL SCREENING

Phytochemical studies of Neem preparations have been revealed as comprising mainly sterols, flavonoids and glycosides (Iwalewa *et al.*, 2005). Tannins, alkaloids, saponins and lectins (Sodipo and Tihze, 2002 and Sadou, 2005; Kamis, 2007).

DETERMINATION OF TANNINS:

Tannin content of the sample was determined by the Felin-Dennis Spectrophotometric method (Pearson, 2006). A measured weight of the sample was dispersed in 50ml of ethanol. The mixture will be shaking for 30 minutes at room temperature and filtered using whatman filter paper. 2ml of the mixture was mixed with equal volume of Felin-Dennis reagent in a 50ml volumetric flask. 2ml of saturated sodium carbonate solution was added. The mixtures were diluted to the 50ml mark and allow incubating for 90 minutes at room temperature. Meanwhile, a standard tannin solution was prepared (with tannin acid) and diluted to a desired concentration. The diluted standard and samples was at 780nm in a spectrophotometer. The tannin content was calculated using the formula below:

$$\% \text{ Tannin} = \frac{100 \times \text{au} \times \text{c}}{\text{W}} \times \frac{\text{vf}}{\text{as}} \times \frac{\text{D}}{1000 \text{ va}}$$

Where,

W = weight of sample analysed

au = absorbance of the test samples

as = absorbance of the standard tannin solution

c = concentration of the tannin solution in mg/ml

vf = Total filtrate analysed

D = Dilution factor where applicable. (Harborne, 2003)

DETERMINATION OF ALKALOIDS

2.20g of the sample was dispersed in 100ml of 10% acetic acid in alcohol solution. The mixture was been shake vigorously and allowed to stand for 3 hours at room temperature and shaking every 30 minutes. The mixture was concentrated by heating to a quarter of its original volume. It was then treated

with drop wise addition of concentrated ammonia solution in precipitate the alkaloids. Ammonia was continuously added until in excess. The precipitated alkaloid was filtered using whatman filter paper. After washing with 1% NH_3OH solution the precipitated alkaloid was dried. The filter paper was also dried and weighed (Sodipo and Tihze, 2002, Sadou, 2005; Kamis, 2007). The alkaloid content was calculated as shown below:

$$\% \text{ Alkaloid} = \frac{(w_2 - w_1)}{\text{Weight of sample}} \times 100$$

W1 = weight of empty filtered paper

W2 = weight of filter paper and alkaloid precipitate

DETERMINATION OF HYDROGEN CYANIDE (HCN)

.18g of the sample was weighed out and 100ml of distilled water added into it. The sample was passed through a Soxhlet Extraction Unit and the cyanide distilled into 10ml of 2.5% NaOH. Distillation was discontinued when about 80ml of distillate was collected. The volume was made up to 100ml with distilled water. 50ml of the recovered sample was measured and titrated with 0.02N AgNO_3 after adding 2ml of 5% potassium iodide and 1ml of 5% NH_4OH . This was done twice at titre values of 0.06 and 0.07 (Harborne, 2003).

RESULTS

The phytochemical analysis of ethanolic Neem (*Azadirachta indica*) seed oil extracts showed the presence of hydrogen cyanide (HCN), Alkaloid and Tannin (Table 4.1).

Table 1: PHYTOCHEMICAL SCREENING OF ETHANOLIC NEEM SEED OIL EXTRACT

PHYTOCHEMICALS	COMPOSITION
Alkaloids	++
Tannins	+
Hydrogen cyanide (HCN)	+

Key: + = present in small amount and ++ = present in moderate quantity

2 MINIMUM INHIBITORY CONCENTRATIONS (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC).

Microorganisms	33.3%	50%	100%	MIC%	MBC%
<i>S.aereus</i>	++	+	-	100	>100
<i>E. col</i>	++	++	+	>100	-
<i>S. typhi</i>	++	+	-	100	>100
<i>P.aeruginosa</i>	++	++	-	100	>100

Key : - No growth, ++ = very turbid with growth, + = Slightly turbid with growth

The minimum inhibitory concentration (MIC) of all isolates shows that *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* showed an MIC of 100%, >100%, 100%, 100% respectively (Table.2).

Minimum bacteriocidal concentration (MBC) was at >100% for *Staphylococcus aureus*, no growth for *Escherichia coli*, >100% for *Salmonella typhi* and *Pseudomonas aeruginosa* was at >100% (Table 2).

The Biochemical characterization (Table 3) shows the results of different tests on the bacterial isolates from clinical specimen.

Table 3 The Biochemical characterization of bacterial isolates obtained from clinical specimens

	Colony Morphology	Microscopic	Characteris	Grain stain	Glucose	Lactose	Slant	Butt	Gas	H ₂ S	Catalase	Methyl red	V.P	Indole	Motility	Oxidase	Coagulase	Sucrose	Glucose	Lactose	Test	Organisms
1	Round cream colony on nutrient agar	Small rods	+	+	+	A	A	-	-	+	+	-	-	-	-	+	-	A	A		<i>S.aureus</i>	
2	Raised cream colony on nutrient agar	Scattered short rods	-	+	+	A	A	+	-	+	+	-	+	+	-	-	A G	A G	A G		<i>E. coli</i>	

3	Round cream yeast colony on nutrient agar	Short rods	-	+	-	B	A	-	-	+	+	-	-	+	-	-	-	A G	-	<i>S. typhi</i>
4	Round cream yeast colony on nutrient agar	Short rods	-	-	-	B	B	-	-	+	+	-	-	+	+	-	-	A	-	<i>P.aeruginosa</i>

Key + = positive reaction, - = Negative reaction, A = Acid production, AG = Acid and Gas production, B = Base production.

Table 4 the zones of inhibition shows that, at 33.3%; *Salmonella typhi* showed the highest zone of inhibition, at 50%; *Salmonella typhi* also showed the highest zone of inhibition, and at 100%; *Pseudomonas aeruginosa* showed the highest zone of inhibition, while *Escherichia coli* showed the least zone of inhibition at all level of concentrations of the extract. Also, the zones of inhibition on the test organisms on Ampicillin control were high, except *Salmonella typhi* which shows the least.

TABLE.4: ZONES OF INHIBITION OF ETHANOLIC EXTRACT OF THE NEEM (*Azadirachta indica*) SEED ON EXTRACT ON FOUR BACTERIAL ISOLATES

Concentration of Extracts in (%)	Zones of inhibitions (mm)			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Pseudomonas aeruginosa</i>
33.3%	3	2	4	2
50%	6	3	9	4
100%	10	9	11	14
Ampicillin control 100%	18	19	15	23



Plate 1 shows the zone of inhibition of *Pseudomonas aeruginosa* (2mm, 4mm and 14mm) on neem (*Azadirachta indica*) seed oil extract at different concentrations (33.3%, 50%, and 100%) respectively. Also, shows the zone of inhibition of 23mm on 100% concentration of Ampicillin control.



Plate 2 shows the zone of inhibition of *Salmonella typhi* (4mm, 9mm and 11mm) on neem (*Azadirachta indica*) seed oil extract at different concentrations (33.3%, 50%, and 100%) respectively. Also shows the zone of inhibition of 15mm on 100% concentration of Ampicillin control.

Plate 3 shows the zone of inhibition of *Staphylococcus aureus* (3mm, 6mm and 10mm) on neem (*Azadirachta indica*) seed oil extract at different concentrations (33.3%, 50% and 100%) respectively. Also shows the zone of inhibition of 18mm on 100% concentration of Ampicillin control.



Plate 4 shows the zone of inhibition of *Escherichia coli* (2mm, 3mm and 9mm) on neem (*Azadirachta indica*) seed oil extract at different concentrations (33.3%, 50% and 100%) respectively. Also shows the zone of inhibition of 19mm on 100% concentration of Ampicillin control (100%).

DISCUSSION

Neem (*Azadirachta indica*) seed oil was capable of suppressing the test organisms such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*. This agrees with the work of (Patel *et al.*, 2002). Biological substances like Azadirachtin, Meliantriol, Salannin, Nimbin and Nimbidin, all of which have the same basic limonoid structure, some of these biological components of neem may be responsible for the reported effects of Neem preparations (National research Council, 2002; Schmulterer and Ascher 2007).

This present study shows that, the observed inhibitory effects increased with increase in concentration of the extract (Table 4). Neem (*Azadirachta indica*) seed oil extract exhibited more inhibitory effect on *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi* and least on *Escherichia coli* (Table 4).

This present study used ethanol for the extraction of the neem (*Azadirachta indica*) seed oil which may have extracted more secondary metabolites which includes; Tannins, Alkaloids and Hydrogen cyanide in Neem oil extract as obtained in this work, this agrees with the work of (Sodipo and Tihze 2002). The weight of seed before extraction was 70g after extraction was 40g and the percentage weight of the extract was 57% (Table 5). The minimum Inhibitory concentration (MIC) and minimum bacteriocidal concentration (MBC) exhibited by the Neem (*Azadirachta indica*) seed oil extract on *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* is of great significance.

All the four test organisms used in this study, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli* showed high sensitivity to ethanolic extract of neem (*Azadirachta indica*) seed oil extract at the highest concentration, while only *Staphylococcus aureus* and *Salmonella typhi* showed high sensitivity to the extract at lower concentrations (33.3% and 50%), corroborating the report of National Research Council (2002). They also showed high sensitivity to the Ampicillin control used.

The preliminary phytochemical screening of the extract of the Neem seed oil confirms the presence of the alkaloids with 48.09%, Tannin 0.362% and Hydrogen cyanide 5.95mg/100g (Table 4.1). These compounds were reported to be an indication of the potential of antimicrobial agents (NRC, 2002).

RESEARCH IMPLICATION

The result so far obtained has shown that the neem seed oil has important medicinal application as many locals use the leaves and stem bark of the plant for malaria treatment while the seed abound and are wasted. The rural population who could not afford orthodox medication could be encourage to patronize the seed of neem for treatment of typhoid and urinary tract infections

CONCLUSION

This present study affirmed that neem (*Azadirachta indica*) possesses some antibacterial activities. It has been revealed that, the oil extract from the seed of the neem tree (*Azadirachta indica*) can help control infections caused by *Staphylococcus aureus* and *Salmonella typhi* effectively. It has also been revealed that the oil is not very efficacious against *Escherichia coli* and *Pseudomonas aeruginosa* except at very high concentration. The oil extract of *Azadirachta indica* on *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* is of great significance more so that, these multi drug resistant organisms have great epidemiological threats. The antibacterial activity of neem (*Azadirachta indica*) seed oil extract in this study is concentration dependent. Therefore, these tests organisms all show susceptibility to ethanolic neem seed oil extract at different concentrations. The orthodox antibiotics are usually accompanied with contra indications while most medicinal plants are

relatively free of such contraindications (Rao, 2006). Therefore, this can be used as an alternative to orthodox antibiotic as it is cheaper and easy to procure.

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