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ANTIMICROBIAL POTENCY OF NEEM (AZADIRACHTA INDICA) LEAF EXTRACTS ON PATHOGENIC BACTERIA ISOLATED FROM WOUND INFECTIONS

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Abstract: The use of herbal decoctions in treating wound infections is increasing especially in developing countries including Nigeria. The increasing incidence of wound infections, coupled with the scarce foreign exchange to import potent antibiotics into the country and the inherent capacity of the wound pathogens to resist the therapeutic effects of such antibiotics have necessitated the use of alternative but effective medical remedies for wound treatment. The objective of this study is to determine the antibacterial activity of Azadirachta indica on common wound pathogens like Pseudomonas aeruginosa Staphylococcus aureus and Streptococcus pyogenes. Agar diffusion assay was employed to determine the antibacterial activity of the ethanolic and aqueous extracts of the neem plant at six concentration levels by measuring the diameter of zones of inhibition around the tested organisms. The data obtained was statistically analyzed using student t-test of unpaired comparison. Streak plate technique was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the tested extracts. Both the ethanolic and aqueous extracts exhibited considerable antibacterial activity against the selected wound pathogens Using agar disc diffusion assay and at varying concentrations, the two extracts showed antibacterial activity against all test bacteria used. When compared, ethanolic extract showed greater zones of inhibition than the aqueous extract. The results showed that sensitivity is directly proportional to the concentrations of the neem extract. It is therefore important that because of the low cost of production of both types of extracts, it should be encouraged as an option in African countries especially Nigeria. This beneficial use of the plant forms a basis for further research and evaluation.

Keywords: Antibacterial potency, neem leaf extracts, Pseudomonas, Staphylococcus Streptococcus, wound infection.

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1. Introduction

Plant extracts or secondary metabolites have served as antioxidants in phytotherapeutic medicines to protect against various diseases for centuries. However, an excess emphasis on the use of chemicals to control infections, have created more problems than addressing them [1] & [2].

Plant extracts, most especially neem, have been shown scientifically to have antibacterial effect [3]. Neem (*Azadirachta indica*), a member of the maliceae family is a fast growing tropical evergreen tree with a highly branched and stout solid stem [4], [5], [6], [7], [8], and [9]. Because of its tremendous therapeutic, domestic, agricultural and ethno medical significance, and its proximity with human culture and civilization, it has being called ‘’the wonder tree’’ and ‘’nature’s drug store’’ [10], [11]. All the part of the tree, particularly the leaves, bark, seed, oil and their purified products are widely used for treatment of cancer, bacterial infections, fungi infections etc [9] &2[11]. Over 60 different types of biochemical including, *Nimbolide*, *Margolone*,

Mahoodin, *Margolonone etc* have been purified from neem [3], [9].

Preclinical research work done during the last decade has fine-tuned the understanding of the antibacterial property of the crude and purified product from this plant [12], [13]. The anticancer properties of the plant has been studied largely in terms of its preventive, tumor-suppressive, immunodulatory and apoptotic effects against various types of cancer and their molecular mechanism [11]. The importance of the neem tree has been recognized by the US National Academy of Science, which published a report in 1992 entitled 'neem-a tree for solving global problems. More than 135 compounds have been isolated from different parts of the plant, and have so far being divided into isoprenoid and non-isoprenoid componds [6], [9] & [14].

The neem tree is known by a variety of names such as Indian lilac (English), Azadirakta (Persian), Mongosa and Neem (Arabic), Tamar (Burmese). Kohomba (Sinhala), Pokoksemembic (Malaysia), Dogonyaro (some Nigeria languages) Neen (Hindu and Banola), Nimba (Sanskrit and Marathi), Arya- veppus (Malyalam), Vaypum (Tomil), Bevu (Kannua) and Nim tree, VepuVempu, Vepa (Telugu). In Southeast Africa it is also known as Mwarobouni (Swahili) which literally means 'the tree of the 40' as it is considered as a treatment for 40 different diseases [11]. It has inherent antibacterial, antifungal, anticancer activities [9], [15] & [16]. This research seeks to assess the antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes, and Pseudomonas aeruginosa isolated* from wound infections. This work also tries *t*o determine the effect of varying concentrations of the neem leaf extract on the growth and survival of the above named microorganisms and serves as a baseline for further research.

2. Materials and method

This features a summary of the basic processes involved in the collection of plant, extraction of the plant, collection of isolates and the necessary microbiological quality assurance. In the case of reagents, the manufacturer's instructions were adhered to strictly.

2.1 Collection of samples

The plants materials used were leaves of *Azadirachta indica* obtained proximal to St. Paul`s Catholic Church, University of Calabar, Calabar on the basis of cost effectiveness and ease of availability.

2.2 Processing of samples

The leaves of the plant weighing 900g were properly washed with tap water and then rinsed in distilled water. The rinsed leaves were dried in an oven at a temperature of 33°C for 3days. The dried leaves of the plants were blended to fine powdered form using manual blender (F No4 Quaker City mill Philadelphia PA USA F8). The powdered form of the plant was stored in air-tight glass container, protected from sunlight until required for analysis.

2.3 Preparation of leaf extracts samples

Batch extraction was then carried out on the dried material. Exactly 100g of the dried powder were taken in a separate container and 625ml ethanol (for ethanolic extract, **EE**) was added and kept for 24hrs with periodic shaking. The mixture was then filtered using No1 whattman filter paper. The procedure was repeated twice with fresh volume of ethanol before the filtrate was pooled. The same procedure was repeated for aqueous extract (**AE**) using cold distilled water.

2.4 Dilution of Extract and preparation of antibiotics disc

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The powdered extract was weighed and dissolved in distilled water as follows; 30mg/ml, 50mg/ml, 100mg/ml, 120mg/ml, 150mg/ml, and 180mg/ml. The same procedure was carried out for the ethanolic extract using distilled water.

Antibiotic discs of equal size, 4mm, were prepared using sterile filter papers. To each of the concentration prepared (30mg/ml, 50mg/ml, 100mg/ml, 120mg/ml, 150mg/ml, and 180mg/ml) for the respective extracts, eighteen (18) paper discs were inserted for an hour (1hr), three in each of the concentration for impregnation or absorption of antimicrobial agent and then removed and air dried after which it was tested for antimicrobial effect as shown below.

2.5 Microbiological analysis

The bacteria used in the study were clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogenes* from wound swabs obtained from the Microbiology and Parasitology Laboratory, University of Calabar Teaching Hospital (UCTH). Biochemical tests were performed to identify the isolates. Fresh cultures of the microorganisms were made from the culture isolates obtained on agar slants (Barrow and Felthan 1993).

The media used were Nutrient agar, Cystine Lactose Electrolyte-Deficient (CLED) agar, MacConkey agar and Mueller-Hinton agar. They were prepared with strict adherence to manufacturer's instructions

The bacteria were inoculated into peptone water and incubated at 37°C for 3hours and was then used as inoculum. A sterile cotton swab was inserted into the bacterial suspension and then rotated and compressed against the wall of the test tube so as to express the excess fluid. The surface of Mueller Hinton Agar plate was inoculated with the swab. To ensure they grow in uniform and confluent (or semi confluent) form, the swab is passed three times over the entire surface by repeating the procedure.

Agar disc diffusion method according to Bauer *et al* (1966) was adhered strictly to. Antibiotic-impregnated disc was placed on agar previously inoculated with the test bacteria; the antibiotic diffuses radially outward through the agar, producing an antibiotic concentration gradient. It was observed that the antibiotic concentration decreases with increase in distance from the sensitivity disc.

3. Results

Table 1, 2 and 3 show the results of the agar disc diffusion method using **EE** and **AE** on *Staphylococcus aureus, Streptococcus pyogenes* and *Pseudomonas aeruginosa* with their zones of inhibition relative to concentrations of the extracts.

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Table1: Zones of inhibition (mm) of varying concentrations of extracts (mg/ml) on the test organisms

Bacteria	Extract	Concentrations					
		180mg/ml	150mg/ml	120mg/ml	100mg/ml	50mg/ml	30mg/ml
S. aureus	EE	12mm	10mm	9mm	8mm	7mm	-
	EA	9mm	8mm	7mm	6.5mm	3mm	-
S. pyogenes	EE	21mm	15mm	14mm	12mm	10mm	10mm
	EA	13mm	10mm	8mm	7mm	6mm	6mm
P. aeruginosa	EE	20mm	15mm	15mm	14mm	10mm	7mm
	AE	12mm	10mm	9mm	8.6mm	7.8mm	6mm

Table 2: Zones of inhibition (mm) of varying concentrations of ethanolic extract (mg/ml) on the test organisms

Bacteria	Extract	Concentrations					
		180mg/ml	150mg/ml	120mg/ml	100mg/ml	50mg/ml	30mg/ml
S. aureus	EE	12mm	10mm	9mm	8mm	7mm	-
S. pyogenes	EE	21mm	15mm	14mm	12mm	10mm	10mm
P. aeruginosa	EE	20mm	15mm	15mm	14mm	10mm	7mm

Table 3: Zones of inhibition (mm) of varying concentrations of aqueous extract (mg/ml) on the test organisms

Bacteria	Extract	Concentrations					
		180mg/ml	150mg/ml	120mg/ml	100mg/ml	50mg/ml	30mg/ml
S. aureus	AE	9mm	8mm	7mm	6.5mm	3mm	-
S. pyogenes	AE	13mm	10mm	8mm	7mm	6mm	6mm
P. aeruginosa	AE	12mm	10mm	9mm	8.6mm	7.8mm	6mm

Key: EE = Ethanolic Extract
AE = Aqueous Extract.

4. Discussion

As the results indicate, the diameter of the zones of inhibition varied. This revealed that the neem leaf extracts inhibited the test bacteria according to [17] who mentioned that inhibition was scored positive if the width of the clear zone around the antibacterial agent or colonies of the producer strain was 0.5mm or larger. It also agrees with the findings of [5] and [18] in that the extracts showed antibacterial activity at all concentrations against *P. aeruginosa, S. aureus and S. pyogenes*. Be that as it may, for the **EE** the sensitivity is slightly greater for *S. pyogenes* than *P. aeruginosa* but largely greater than *S. aureus*. Whereas for **AE** the sensitivity is relatively the same for *S. pyogenes* and *P. aeruginosa* but lower for *S. aureus*. The results also show t0hat as the concentrations of the extract increases there is a corresponding increase in the zones of inhibition and comparing the activity, ethanolic extract of neem leaf exhibited higher antimicrobial activity against the test bacteria than aqueous extract. This difference may be due to the inhibitory ability of the ethanol even without extract, whereas the ability of the aqueous extract to exhibit this inhibition may be primarily due to its penetrative ability and the compositions of neem such as *Margolone*, *Margolonone*, *Isomargolonone*, *Azadirachtin*, *Quercetin*, etc. that are well distributed in the leaf of *A.indica* [5], [6], [15] & [19], [20]The antibiotic potentials are actually the power and defensive mechanism of the plant against different pathogens [21].

5. Conclusion

On the basis of the results obtained from the study, the antimicrobial potential of neem leaf extracts is effective and most of the phytochemicals are responsible for its high therapeutic value. The study also confirmed antimicrobial properties of neem leaf. The results of this study have shown that neem leaf can be exploited for the treatment of wound infections caused by these bacteria. This beneficial use of the plant forms a basis for further research and evaluation.

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