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**Antimicrobial Activity of Neem (*Azadirachta indica* A. Juss.) Aqueous
 and Methanolic Extracts against Pathogenic Bacteria (*Staphylococcus
 aureus*) and Fungi (*Candida albicans*)**

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ABSTRACT

Plants have many biologically active compounds which have latent for development as medicinal agents. Neem extracts and its different constituents play essential role in the inhibition of several microbes. Neem (*Azadirachta indica*), the versatile medicinal plant is the source of several compounds having diverse chemical structure and biological effects. The research aimed to evaluate the antimicrobial activity of neem tree aqueous and methanolic extracts against pathogenic fungi and bacteria. It was conducted in the month of August 2021 at Microbiology laboratory, Federal University Gusau. The neem plant samples were collected behind Department of Biological Sciences of Federal University Gusau, Zamfara State. The plant sample were identified and authenticated in the herbarium of the Department of Biological Sciences, Federal University Gusau. The samples were shade dried and grounded in to powder using mortar and pestle. The aqueous and methanolic extracts of the plant samples were prepared using Soxhlet apparatus. Concentrations of 50mg/ml, 100mg/ml and 150mg/ml were prepared and used against *Staphylococcus aureus* and *Candida albicans*. The results showed highest zone of inhibition in 150mg/ml and 100mg/ml and low zones of inhibition in 50mg/ml in aqueous and methanolic extracts of neem parts. It could be concluded that, neem parts of both aqueous and methanolic extracts showed effectiveness against *Staphylococcus aureus* and *Candida albicans* in the composite concentrations, therefore, the plant can be used as a source of crude drugs in the production of synthetic drugs that can be used in the treatment of fungal and bacterial diseases.

Keywords: Antimicrobial, *Azadirachta indica*, *Candida albicans*, Neem, *Staphylococcus aureus*.

1.0 INTRODUCTION

Neem (*Azadirachta indica*) belongs to family Meliaceae, originated from South and Southeast Asia, also found in tropical and subtropical areas of Africa, America and Australia. In India, it is commonly known as 'Divine Tree', 'Life-giving tree', 'Nature's Drugstore', 'Village Pharmacy' and 'Panacea for all diseases' Products made from neem trees have been used in India for over two millennia for their medicinal properties (Shukla and Bhalodia, 2011). Neem tree has various biological activity such as anti-inflammatory, antiarthritic, antipyretic, hypoglycaemic, antigastric ulcer, anti-tumour, immunomodulatory etc (Biswas *et al.*, 2015). Neem oil and the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, and constipation and also as a general health promoter. Its use for the treatment of

rheumatism, chronic syphilitic sores and indolent ulcer has also been evident (Muriana *et al.*, 2017).

Neem is effective in treating eczema, ringworm, acne, anti-inflammatory, anti-heperglycemic properties and it is used to heal chronic wounds, diabetic food and gangrene developing conditions (Jigna *et al.*, 2017). It is believed to remove toxins from the body, neutralize free radicals and purify the blood. It is used as anticancer agent and it has hepato-renal protective activity and hypolipidemic effects. A wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. which have been found in vitro to possess medicinal properties. Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds. Phytochemicals from medicinal plants serve as lead compounds in antimicrobial discovery

(Maria and Romily, 2017). The widespread use of a limited number of antimicrobial agents concomitantly with the reduced arsenal of drugs with antimicrobial function, has led to the development of resistance to drugs that oppose both fungal and bacterial infections, which has been an increasing problem. The need to find alternatives for microbial control has induced a lot of research in order to seek products that are effective and economical. Studies of natural products with biological activity offers credible alternatives for microbial control, particularly from bioactive products derived from plants with therapeutic properties of routine use (Gejalakshni *et al.*, 2020).

The medicinal properties of the plant were studied by several workers. The antipyretic effect antimalaria effect antidiabetic effect, antifertility effect, effect on the central nervous system cardiovascular effect and wound healing were some of the studies of earlier workers. *A. indica* has been shown to possess antimicrobial properties by several studies. Several studies on neem reported the antimicrobial activity of the seed oil against a variety of pathogens. Oils from the leaves, seeds and bark possess antibacterial action against certain bacteria. Extracts of neem leaf, neem oil and seed kernels are effective against certain human fungi (Itelima *et al.*, 2016). The purpose of this study was to investigate the antimicrobial activity of Neem (leaf, bark and root) against pathogenic bacteria (*Staphylococcus aureus*) and fungi (*Candida albicans*). This research would provide information

on the effectiveness of neem tree part on fungi and bacteria in the study area.

2.0 MATERIALS AND METHODS

2.1 Study Area

This research was conducted at Microbiology Laboratory of Federal University Gusau, Zamfara State Nigeria, Gusau is the capital of Zamfara State which is located in North-western Nigeria. It has a Latitude 12° 11 North and Longitudes 6° and 14° East, with a total land Area of 39,762km². The climatic condition of Zamfara State is 38°C (100.4°F) and above between March to May. Rainy season starts in late May to September while the cold season known as Hamattan lasts from December to February (Abdu and Swendell, 2020).

2.2 Sample Collection and Preparation

Fresh leaves, barks, and roots of *A. indica* was collected behind Department of Biological Sciences of Federal University Gusau, Zamfara state, Nigeria. The plant samples were identified and authenticated by a taxonomist and also in charge of the Herbarium of the Department of Biological Sciences Federal University Gusau. The voucher number obtained is FUG/BIO/HERB/21/0086. After collection, the samples were shade dried for two weeks, the dried samples were grounded in to powder with aid of mortar and pestle, the powder were used for the analysis.



Plate i: Neem tree (*Azadirachta indica*) growing behind Department of Biological Sciences, Federal University Gusau, Zamfara State.

2.3 Determination of Antimicrobial Activities of Neem Parts

2.3.1 Preparation of Ethanol Neem Extracts

Extraction was carried out by soaking 15g of the neem powder into 100ml of the distilled water in a round bottom flask with occasionally shaking the extract the mixture was allow to stand for 48hr at room temperature the mixture was filtered using muslin cloth, the filtrate was extracted using rotary evaporator (EdrisAli *et al.*, 2021).

2.3.2 Preparation of Methanol Neem Extracts

Extraction was carried out by soaking 15g of the neem powder into 100ml of methanol in a round bottom flask with occasionally shaking the extract the mixture will allow to stand for 48hr at room temperature. The mixture was filtered using muslin cloth, the filtrate was extracted using rotary evaporator (Mahmoud *et al.*, 2011).

2.3.3 Test Organisms

The organisms used in the experiment were *Candida albicans* (Fungi) and *Staphylococcus aureus* (gram positive bacteria). The test organisms were obtained from Microbiology Laboratory of Department of Microbiology, Federal University Gusau, Zamfara State Nigeria.

2.3.4 Preparation of Inoculum Suspension

The test organisms were suspended with sterile loop in to sterile test tubes containing sterile normal saline and the mixed thoroughly. The suspensions were matched with 0.5 McFarland turbidity standards. The stock cultures of the bacteria used were sub-cultured at 37°C for 24hrs (Maria *et al.*, 2017).

2.3.5 Antimicrobial Assay

Method of Ugwu, 2019 was adopted. The antimicrobial assay of aqueous and methanol extracts of Neem leaves, bark and roots were performed by agar well diffusion method. To test antibacterial activity of Neem extracts, 0.8 g of each extract was dissolved in DMSO and then varying concentrations of the extracts (50mg/ml, 100mg/ml, 150mg/ml) were obtained. A standard inoculum of 1.5×10^8 cells which matched 0.5 McFarland standard was spread on the surface of sterile Muller Hinton agar plates in duplicates. A sterile 6 mm cork borer was used to make a hole on the Muller Hinton agar plates in which 0.1 ml of each of the plant extracts were added. The plates were incubated at 37°C for 24 h. The

antimicrobial activity was detected by measuring zones of inhibition in millimetres.

3.0 RESULTS AND DISCUSSION

Results on antimicrobial activity of aqueous extracts of neem leaf extracts against *Staphylococcus aureus* showed the highest zone of inhibition of 25 mm on 150 mg, followed by 19 mm on 100 mg, the lowest was on 50mg with 16 mm. The bark aqueous extracts showed the highest zone of inhibition of 20 mm on 150mg, followed by 19 mm on 100mg, the lowest was in 50mg with 13 mm. The root aqueous extracts showed the highest zone of inhibition of 13 mm on 150mg, followed by 11mm on 100 mg, the lowest was on 50 mg with 0 mm zone of inhibition. Methanolic leaf extract against *Staphylococcus aureus* showed the highest zone of inhibition of 21mm on 150 mg, followed by 20mg on 100mg, the lowest was on 50 mg with 18 mm. Bark methanolic extract showed the highest zone of inhibition of 20mm on 150mg, followed by 18mm on 100mg, the lowest was on 50mg with 14mm. Root methanolic extract showed the highest zone of inhibition of 19mm on 150mg, followed by 16mm on 100mg, the lowest was on 50mg with 13mm (Table 1).

Antimicrobial activity of aqueous neem extracts against *Candida albicans* showed the highest zone of inhibition of 18mm on 150mg, followed by 16mm on 100mg, the lowest was on 50mg with 10mm. Bark aqueous extract showed the highest zone of inhibition of 20mm on 150mg, followed by 19mm on 100mg, the lowest was on 50mg with 15mm. The root aqueous extract showed the highest zone of inhibition of 16mm on 150mg, followed by 15mm on 100mg, the lowest was on 50mg with 10mm. Methanolic neem leaf extract against *Candida albicans* showed the highest zone of inhibition of 20mm on 150mg, followed by 18mm on 100mg, the lowest was on 50mg with 13mm. Methanolic bark extracts showed the highest zone of inhibition of 22mm on 150mg, followed by 20mm on 100mg, the lowest was on 50mg with 18mm. The root methanolic extract showed the highest zone of inhibition of 13mm on 150mg, followed by 11mm on 100mg, the lowest was on 50mg with 0mm (Table 2).

The results obtained from aqueous and methanolic extracts of the leaf, bark and roots of neem parts showed the highest zone of inhibition (mm) on 150mg/ml and 100mg. It was observed that, root

extracts showed the lowest zone of inhibition at all the concentrations, this might be as a result of low bioactive compounds present in the leaves and nature of the solvents used during extraction process. The low zone of inhibition observed in root extracts indicates the low effectiveness against *Staphylococcus aureus* and *Candida albicans*, this finding agrees with findings of Anannya et al. (2014) who worked on antimicrobial activity of neem (*Azadirachta indica*) leaf and root extracts and observed low zone of inhibition in root extracts against *Escherichia coli* and *Staphylococcus aureus*. Furthermore, the results of this study showed that, the effectiveness of extracts was dependent of concentration used, thus, increase in extracts

concentration leads to increase the zone of inhibition. *Azadirachta indica* used in this research possessed a good anti-microbial activity, confirming the great potential of bioactive compounds and is useful for rationalizing the use of this plant in traditional medicine. This result was in accordance with work of Gauri et al. (2018), who worked on antibacterial activity of mixture of leaf extracts of neem (*Azadirachta indica* and Tantani (*Lantana camara*) and found the highest zone of inhibition against pathogenic microorganisms at 10mg/ml, 50mg/ml and 100mg/ml respectively. The extracts of Neem when used as medicinal plant, could be useful for the growth inhibition of the pathogenic fungi and bacteria (Maragathavalli et al., 2012).

Table 1: Antimicrobial Activity of Neem Extracts Against *Staphylococcus Aureus*

CONC	AQUEUOS			METHANOL		
	50mg	100mg	150mg	50mg	100mg	150mg
	Zone of inhibition (mm)					
EXTACTS						
LEAF	16	19	25	18	20	21
BARK	13	19	20	14	18	20
ROOT	0	11	13	13	16	19

Table 2: Antimicrobial Activity of Neem Extract Against *Candida Albicans*

CONC.	AQUEUOS			METHANOL		
	50mg	100mg	150mg	50mg	100mg	150mg
	Zone of inhibition (mm)					
EXTACTS						
LEAF	10	16	18	13	18	20
BARK	15	19	20	18	20	22
ROOT	10	15	1	0	11	13

4.0 CONCLUSION AND RECOMMENDATION

It could be concluded that, results obtained from this study showed higher zone of inhibition against *Staphylococcus aureus* and *Candida albicans* at varying concentration of both aqueous and methanolic leaf extracts of neem leaf and bark extracts, the root extracts showed lower zone of inhibition in all the concentrations used. Therefore, neem tree parts can be used in traditional medicine in the treatment of fungal and bacterial diseases.

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