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Antimicrobial Properties of Ethanol Leaf Extract of *Millettia aboensis* on Some Selected Clinical Isolates

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Abstract There is renewed interest in the search for plants with anti-microbial activity leading to various plants being investigated for their potential efficacy. Ethanol leaf extract of *Millettia aboensis* and reference drug (commercial Geniclox 500) were tested in vitro against four clinical isolates, namely, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus* for antibacterial activity. The ethanol leaf extract of *M. aboensis* inhibited the growth of *K. pneumonia*e, *P. aeruginosa*, and *S. aureus* but did not inhibit the growth of *E. coli* while the reference drug inhibited the growth of all the isolates. The Minimum inhibitory concentration (MIC) against susceptible organisms for the ethanol extracts was 12.5mg/ml for *K. pneumoniae* and *P. aeruginosa* and 50 mg/ml for *S. aureus*. The MIC of the reference antibiotics was 12.5 mg/ml for *E. coli*, *P. aeruginosa* and *K. pneumonia*e and a higher concentration of the reference antibiotics (200 mg/ml) was needed to inhibit *S. aureus* when compared to *E. coli*, *K. pneumoniae* and *P. aeruginosa*. The equivalent-point inhibitory kinetic (I_{keq}) for both the reference drug and solvent extract of *Millettia aboensis* against *P. aeruginosa* and *K. pneumoniae* were 125 and 83.33 mg/ml respectively. There is need to harness the efficacious potentials of plants in view of isolating and identification of active principles present in plant extracts which could possibly be exploited for pharmaceutical use.

Keywords *Millettia Aboensis,* Antibacterial Activity, Clinical Isolates, Minimum Inhibitory Concentration, Inhibitory Kinetic

1. Introduction

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in Asia and Africa, but it is without a doubt an art as old as mankind. Traditional medicine is the main source of medical care for a great proportion of the population of the developing world. In Africa, indigenous plants play an important role in the treatment of a variety of diseases [1]. Nigeria untapped forest reserved is rich in diverse forms of life especially medicinal plants which are very usefully in both curative and preventive of various forms of chronic diseases.

Millettia aboensis are small trees of 30–40 feet high and up to 2 feet in girth but usually 12 m high with reddish-brown pubescence on the petioles, branches, inflorescence and fruits. They are found commonly in low land rain forest. The flowers are purple in erect woody racemes up to 18 in. long. It has conspicuously rusty-hairy leaves and handsome purple flowers in erect terminal racemes at branch [2]. Almost all the part of *Millettia aboensis* has medicinal properties. The leaf is used by traditional herbalist for general healing including ulcer healing and laxatives while the root is used in treating gastro intestinal disturbances and liver disease. Also the leaf, stem and roots mixed with other plant materials (herbs) is used to cure veneral diseases such as gonorrhea, syphilis etc[2].

Microbial infections such as tuberculosis, candidiasis, cryptococcosis and salmonellosis are some of the infections that have been on the increase partly due to both environmental and human factors. The successful use of any therapeutic agent is compromised by the potential development of tolerance or resistance to that compound from the time it is first employed[3]. This is true for agents used in the treatment of bacterial, fungal, parasitic, and viral infections and for treatment of chronic diseases such as cancer and diabetes; it applies to ailments caused or suffered by any living organisms, including humans, animals, fish, plants, insects, etc. A wide range of biochemical and physiological mechanisms may be responsible for resistance[3] .

Use of plant products for the control of human diseases has certain advantages besides being cheap to produce; they are biodegradable and readily available. Effective plant extracts can combat human pathogenic bacteria without toxic side effects and environmental hazards[4]. There is renewed

interest in the search for plants with anti-microbial activity leading to various plants including *Azandira chtaindica, Camelia sinensis, hypericumper foratum, Allium sativum* among others being investigated and, they displayed considerable antibacterial activity[4]. Many of the medications currently available to physicians have a long history of use as herbal remodels including aspirin, digitalis and quinine, opium[5]. As medicinal plants are suitable alternatives for synthetic and chemical drugs; they are considered to be full of secondary metabolites as essential oils, antibacterial, antifungal and other products[6,7]. Today according to the World Health Organization reports, as many as 80% of the world's people depend on traditional medicine for their primary health care needs. There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal spices for the treatment of various diseases[8]. Herbal drugs are prescribed widely because of their effectiveness, fewer side effects, and are relatively low in cost[9].

Considering the vast potentiality of plants as sources for alternative drugs with reference to synthetic agents, a methodical investigation was embark on to screen a local plant (*Millettia aboensis)* for possible antimicrobial activity. *Millettia* classified in the family of *fabaceae* is comprised of different species, of which *aboensis* is much common in forest zones of Nigeria, Equatorial Guinea and Cameroun. The species are distinguished based on colour, thickness of vine, size of leaf, growing vigour, days to flowering and succulence. In the forest zone of Nigeria, Equatorial Guinea and Cameroun the specie *Millettia aboensis* are characterized by streaked dark reddish or chocolate-coloured wood, purple[10]. They are mostly tropical trees or shrubs yielding slowly streaked dark reddish or chocolate colour wood. Interaction with local traditional healers on their knowledge and usage of *Millettia aboensis* reveals that the leaf extract is useful in the management of sexual transmitted disease; it is also abortifacients if taken in combination with other herbal plant in excess by pregnant women. The stem extract is also used as a laxative in both children and Adult. In addition, *Millettia aboensis* is used in management of oedema in pregnant women, fever, and malaria locally.

2. Materials and Methods

2.1. Collection and Identification of Plant Material

Fresh leaves of *Millettia aboensis* were collected from a forest at Aluu town in Rivers State, Nigeria The plant was identified and authenticated by Dr Eddy Wosu. A specimen was deposited at the department of Plant and Science Biotechnology for reference purposes with **accession** number UPH 587.

Fresh leaves of *Millettia aboensis* were thoroughly washed using running water and rinsed with distilled water. The leaves were air dried to a constant weight and milled to a fine powder with the aid of a Marlex Exceller grinder (Mumbai, India).

2.2. Solvent Extraction

200 g of the dried fine powdered of *Millettia aboensis* leave was weighed and filled in thimble and extracted using 60% ethanol solvent in Soxhlet extractor. The solvent extract was then concentrated by evaporating to dryness using rotary evaporator at a temperature 40^{o}C. A dark- green coloured mass was obtained and stored in airtight bottles at 4^{o}C in a refrigerator until ready for use.

2.3. Preparation of Test Organisms

Clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from the Department of Medical Microbiology of University of Port Harcourt Teaching Hospital. The isolates were maintained on nutrient broth at 37^{o}C for 8 h prior to antibacterial testing.

2.4. Standard Reference Drug

Geniclox 500 (Ampicillin and Cloxacillin) Injection 500mg was purchased from E-blend pharmacy at University of Port-Harcourt, Abuja Campus. 500mg/ml of the drug in 4ml of water of injection was used as standard reference drug for the *in vitro* tests.

2.5. Antibacterial Sensitivity Testing

Agar well diffusion technique as described by [11] was used to determine the antibacterial activity of the extracts[11] . An 18 mL of Mueller Hinton agar plates (MHA Difco, France) were seeded with 0.1 ml of an overnight culture of each clinical isolate (equivalent to $10^{7} – 10^{8}$ CFU ml^{-1}). The seeded plates were allowed to set after a uniform distribution of the bacterial isolate following slow rotation of the Petri dish.

A standard sterile cork borer of 6mm diameter was used to cut six uniform wells on the surface of Mueller Hinton Agar. The wells on each plate where then filled with the aid of a sterile Pasteur pipette with 0.3 ml of different concentration of the ethanol extract i.e. (12.5mg/ml to 400mg/ml). The dishes were allowed to stand for 45 min at room temperature to allow proper diffusion of the extract to occur. The control experiments were setup with 0.3 mL of ethanol (60%) and distilled deionised water which served as controls were also put in separate wells. All the plates were incubated at 37^{o}C for 24 h. The effect of the extract and the reference drug on the growing ‘lawns’ of each clinical isolates were monitored at intervals of 24 hours. Zones of clearance round each well means inhibition and the diameter of such zones was measured in millimetre (Mm). The minimum inhibitory concentration (MIC: The lowest concentration of the extract at which the growth of bacterial are inhibited) in mg/ml was determined by comparing the different concentration of the

extracts that have different zones of inhibition and then selecting the lowest concentration of each extract[12,16].

The rate of inhibitory (R inhibitory) is defined as the ratio of diameter of zone of clearance (Mm) and time taken to established clearance.

$$\frac{\text{Zones of clearance(Mm)}}{\text{Time (Min)}}$$

The inhibitory kinetics (I_k) defined as the plot of reciprocal of Inhibitory against the reciprocal of varied concentration of the extract and the reference drug (Geniclox), while the equivalent-point on inhibitory kinetic (I_{keq}) is defined as the effective concentration at which both the reference drug and the extract of *Millettia aboensis* show inhibitory activity.

3. Results

3.1. Antibacterial Activity of the Ethanol Extracts of *Millettia Aboensis*

Table 1 shows the mean zone of inhibition of ethanol extract of the leaves of *Millettia aboensis* and the reference drug – Geniclox 500against the clinical isolates of *E. coli, S. aureus*, *P. aeruginosa* and *K. pneumoniae*. The degree of inhibition on the survival of the susceptible clinical isolates showed a varied efficacy of the extract, the extract of *Millettia aboensis* has no growth inhibitory effect on *E. coli* at various concentrations tested. The inhibitory effect of the extract was manifested in *S. aureus* at a 50mg/ml of crude extract concentration, while the reference drug was inhibitory to *S. aureus* at 200mg/ml. Both *P. aeruginosa* and *K. pneumoniae* were susceptible to extract and reference drug at all the various concentration tested.

Table 1. Mean zone of inhibition (MZI) of Ethanol extract of leaves of *Millettia aboensis* and Reference drug against clinical Isolates of *E. coli, S. aureus, P. aeruginosa* and *K. pneumoniae*

Source of antibiotics	Concentration of extract (mg/ml)	Mean zones of inhibition (mm)			
		E.coli	*P. aeruginosa*	*K.pneumoniae*	*S. aureus*
Ethanol Extract of Millettia Aboensis	12.5	Nil	3.1±1.55	4.5±2.14	Nil
	25	Nil	3.6±1.80	5.05±2.42	Nil
	50	Nil	4.1±2.05	5.6±2.68	4.05±0.07
	100	Nil	4.55±2.28	6.1±2.92	4.6±0.14
	200	Nil	5.1±2.55	7.2±3.46	5.1±0.14
	400	Nil	5.65±2.83	8.1±3.90	6.05±0.07
Reference Antibiotic (Genicolox 500)	12.5	3.23±1.47	1.1±0.56	1.15±0.57	Nil
	25	4.23±1.92	2.1±1.05	3.2±1.56	Nil
	50	5.6±2.59	3.05±1.53	5.3±2.65	Nil
	100	7.38±3.35	4.1±2.05	6.1±2.95	Nil
	200	8.45±3.84	5.15±2.58	8.15±3.99	1.2±0.28
	400	9.38±4.24	7.3±3.66	10.25±4.92	2.15±0.21

Results of the minimum inhibitory concentration (MIC) are summarized in Table 2. MIC of the ethanolic extracts was 12.5mg/ml for *K. pneumonia*e and *P. aeruginosa* and 50 mg/ml for *S. aureus*. The MIC of the reference antibiotics was 12.5 mg/ml for *E. coli*, *P. aeruginosa* and *K. pneumonia*e. Higher concentrations of the reference antibiotics (200 mg/ml) were needed to inhibit *S. aureus* when compared to *E. coli*, *K. pneumoniae* and *P. aeruginosa*.

Table 2. Minimum inhibitory concentrations (MICs) of Ethanol extract of leaves of *Millettia aboensis* against the clinical isolates of *E. coli, S. aureus, P. aeruginosa* and *K. pneumoniae*.

Source of antibiotics	Minimum inhibitory concentrations (mm/ml)			
	E.coli	*P. aeruginosa*	*K .pneumoniae*	*S. aureus*
Ethanol extract of Millettia aboensis	Nil	12.5	12.5	50
Reference antibiotic (Geniclox 500)	12.5	12.5	12.5	200

Figures 1 and 2 shows the inhibitory kinetic (I_k) of both solvent extract of *Millettia aboensis* and the reference drug (Geniclox) on *S. aureus*, *P. aeruginosa* and *K. pneumoniae*. The result shows a positive inhibitory correlation between the ethanolic extract of *Millettia aboensis* and the reference drug on both *P. aeruginosa* and *K. pneumoniae*. However, Figure 3 shows a negative correlation on the inhibitory effect of the reference drug and the solvent extract on *S. aureus*.

The equivalent-point inhibitory kinetic (I_{keq}) for both the reference drug and solvent extract of *Millettia aboensis* as extrapolated from the reciprocal plot of concentration and rate of Mean zones of inhibition (Inhibitory) for *P. aeruginosa* and *K. pneumoniae* were 125 and 83.33 mg/ml respectively. While the equivalent-point inhibitory kinetic (I_{keq}) for *S.auerus* gave a negative correlation.

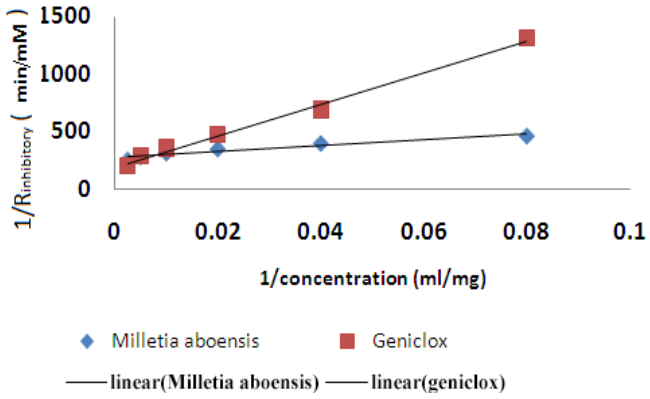

Figure 1. Inhibitory kinetics of *Millettia aboensis* and Reference drug (Geniclox) on *P. aeruginosa*

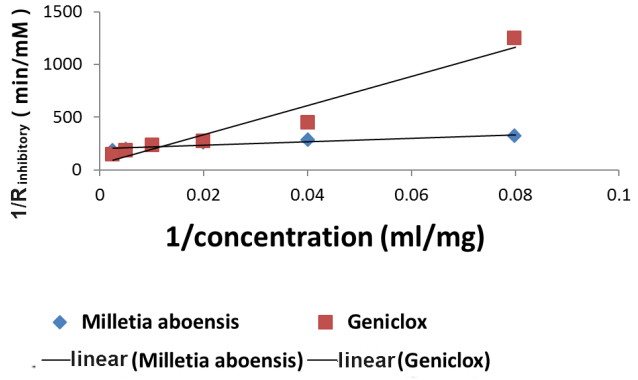

Figure 2. Inhibitiory kinectics of *Millettia aboensis* and Reference drug (Geniclox) *K. pneumoniae*

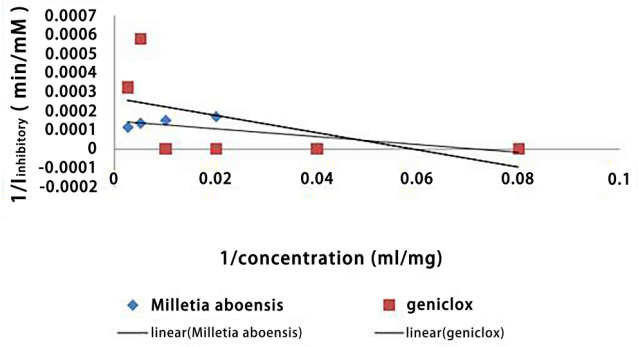

Figure 3. Inhibitiory kinectics of *Millettia aboensis* and Reference drug (Geniclox) on *S. aureus*

4. Discussion and Conclusion

Advent of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search of new antimicrobial drugs of plant origin[17]. Nature is a unique source of structures of high phytochemical diversity, many of them possessing interesting biological activities and medicinal properties. Our previous study showed that the phytochemical component of *Millettia aboensis* is rich in flavonoids, tannis, saponins, and cardiac glycosides[18]. The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments but several studies have also reported various types of contamination of herbal medicines which include microorganisms and toxins produced by microorganisms, pesticides and toxic heavy metals[19]. The ethanol extracts of *Millettia aboensis* exhibited significant antibacterial activity against clinical Isolates of *S. aureus*, *P. aeruginosa* and *K. pneumonia*e at 12.5mg/ml – 400mg/ml concentrations, but not on E. coli. The ethanol extract showed a concentration dependent gradient decrease in the level of inhibition against isolates. The observed difference with the ethanol extracts may be due to solubility of active compounds in ethanol or inhibitors to the antimicrobial component [13].

[14] and [15] reported that inactivity of plant extracts may be due to age of plant, extracting solvent, method of extraction and time of harvesting of plant materials [14,15].

From the results there is variation in the degrees of antibacterial activities of the extracts on the clinical isolates. Ethanol extract of *Millettia aboensis* showed antibacterial activity against *S. aurus* at an MIC of 50mg/ml. This can be deduced to the ability of ethanol to extract more of the essential oils and secondary metabolite which are believed to exert antibacterial activity on the test organism. This study however can justify the use of the leaves in traditional medicine practice as a therapeutic agent against some microbial infection and can explain the long history use of this plant.

Ethanol extracts of *Millettia aboensis* did not inhibit the growth of *E. coli* while the reference antibiotic showed antibacterial activity against *E.coli* even at the lowest concentration of 12.5 mg/ml. This result is in agreement to [12] where it was reported that gram negative bacteria are more resistant than gram positive bacteria to the essential oil which are antimicrobial agents. E. coli contains a high level of lipid material[12]. These materials are thought to make a substantial contribution to the mechanism whereby injurious chemicals are prevented from reaching their sites of action within the cell.

Our results suggest that *Millettia aboensis* can serve as potential source of bioactive healthy compounds in the diet and their consumption could be useful in the prevention of diseases. It can be concluded that the ethanolic extract of *Millettia aboensis* investigated, have opened up a new perspective in pharmaceutical research and they can be used for the development of potential, novel antimicrobial agents for the treatment of clinical microbial isolates related infection. In this bearing, *Millettia aboensis* can be further

investigated as a promising source of antibacterial activity. Further research is needed toward isolation and identification of active principles present in the extracts which could possibly be exploited for pharmaceutical use.

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