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The Antimicrobial Potency of *Anogeissus Leiocarpus* Root Extracts.

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ABSTRACT

In most parts of Nigeria, traditional medicine practitioners and most rural dwellers use plant parts for treatment of ailments without scientific proof. This study was designed to investigate the antimicrobial activities of the aqueous and ethanol root extracts of *Anogeissus leiocarpus* against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 10145, *Bacillus subtilis* NCTC 8236, *Staphylococcus aureus* ATCC 25923, *Candida albicans* 24433 and clinical isolates of *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Klebsiella spp* and *Candida albicans*. The antimicrobial assays were carried out using the agar ditch diffusion and agar well diffusion methods. Statistical analysis was done using two way anova without replication. The ethanol extract inhibited all organisms and had lower minimum inhibitory concentrations (MIC) compared to the aqueous extract ($P < 0.05$). *Staphylococcus aureus* recorded the lowest MIC in ethanol and aqueous extract while *Klebsiella spp* had the highest MIC in ethanol extract. The aqueous extract had no activity against *Candida albicans* ATCC 24433. The use of this plant root alone and in combination with other plants by herb medicine practitioners for the treatment of infectious diseases can therefore be justified. However further work should carried out to isolate the active component that furnishes the understudied plant with its antimicrobial potential and as well determine the toxicity of the plant.

Keywords: Antimicrobial activity, Minimum Inhibitory Concentration, *Anogeissus leiocarpus* and Ciprofloxacin.

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INTRODUCTION

Man in primitive days and even today use plants for the treatment of a variety of human and animal diseases. There are about 200,000 to 300,000 medicinal plants found in developing countries of Africa. Eighty five percentage of Indians and 80% of world population use higher plants as effective antimicrobials for the cure of ailment [1,2,3]. The United Nations encourages the use of herbal medicine of proven safety and efficacy in their healthcare delivery program [4,5]. Plant materials have provided the model for 50% Western drugs (Robbers, 1996). Many modern day drugs have their origin in herbal plants[6]. The therapeutic potency of some medicinal plants is attributed to their constituents that are unique to particular species and family as revealed by phytochemical analysis. Various publications have reported the antimicrobial activities of some of such plant extracts [7,8]. *Zanthoxylum zanthoxyloides* antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Neisseria catarrhalis, Lactobacillus acidophilus, Pseudomonas aeruginosa, Klebsiella rhinosceromatis* and *Candida albican* was reported by [9]. Back and green tea extracts inhibited the growth of enteric bacteria [10]. *Alcalypha wilkesiana* leaves extract was active against *Staphylococcus aureus*, *Candida albican* and *Aspergillus flavus*[11]. The development of antimicrobial resistance to modern and synthetic drugs, high cost of purchase and unavailability of the drugs have led Researchers to start investigating plants used traditionally for the prevention and treatment of infectious diseases [12,13]. *Anogeissus leiocarpus* belongs to the Phylum, Tracheophyta; Order, Myrtales and Family, Combretaceae. It is commonly called African Birch and Axlewood. In Nigeria it is known as Marke and Kwankila in Hausa, Kojoli in Fulani, Annum in Kanuri, kukunchi in Nupe, Otra in Idoma, Atara in Igbo and Orin-Odanainy in Yoruba [14,15,12]. It is a tropical tree that can grow up to 30 m, but typically 15-18 m and occurs mostly in the savannah area to the borders of the forest zone. The plant extends from Senegal in West Africa to Sudan and Ethiopia in East Africa. The roots, stem bark and root bark is widely used in Northern, Eastern and Western Nigeria by traditional medicine practitioners for the treatment of gonorrhea, diabetes, tooth ache, general body pain, wound and ulcer, asthma, cough, tuberculosis and also used as blood clotting agent, acaricide and antihelmintic[16,17,18,19,12]. In Eastern part of Nigeria, the leaves decoction is used externally for the treatment of skin diseases and the itch of psoriasis. The powdered bark mixed with green clay has been used as unusual face mask to treat serious blackheads [16]. It is also used as a vermifuge and the leaves decoctions is also used for washing and fumigation [20]. The root is used as chewing stick [21]. In Ivory Coast the decoction of the leaves is applied to the skin to alter pigmentation and as eye-wash for certain complaints. The leaves and roots with other drug-plants are taken for leprosy. The bark is used as a febrifuge in hot lotions and

infusion in some regions of Upper Guinea [22]. However, the knowledge of *Anogeissus leiocarpus* and its medicinal benefit is limited in Southern Nigeria. It is therefore the aim of this study to investigate the antimicrobial activity and the minimum inhibitory concentration of the aqueous and ethanol root extracts, comparing same with Ciprofloxacin with a view of recommending its use.

MATERIALS AND METHODS

Plant extract

Anogeissus leiocarpus roots were bought from a Yoruba herbal plant seller in Lagos street market, Benin City, Edo state. The roots were authenticated by Professor M. Idu of the Department of Plant Biology and Biotechnology, University of Benin, Benin City. They were washed in water, cut into smaller sizes and air dried, before grinding to powder. Two hundred grams of the powdered sample was macerated in 1L of ethanol and another 200 g in 1.5 L of distilled water for 72 hours, shaking at intervals. They were both filtered and concentrated at 60^{o}C. Concentrations of 800 mg/ml to 0.2 mg/ml for ethanol extract, 800 mg/ml to 2 mg/ml for aqueous extract were made in sterile distilled water.

Ciprofloxacin

Analytical grade Ciprofloxacin, Fluka WA 19781 greater than 98% (HPLC), Lot & Code 1298025,43207340 (85721-33-1) was used as the standard drug. Concentrations of 750 µg/ml to 0.07 µg/ml were prepared in 0.2% dimethyl sulpoxide (DMSO).

Microbial Isolates

Cultures of *Bacillus subtilis* NCTC 8236, *Escherichia coli* ATTC 25922, *Pseudomoas aeruginosa* ATTC 10145, *Staphylococcus aureus* ATTC 25923 and *Candida albicans* ATCC 24433 were obtained from the Department of Pharmaceutical Microbiology, University of Benin and Clinical isolates of *Klebsiella species, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis* and *Candida albicans* were collected from the Department of Medical Microbiology Laboratory, University of Benin Teaching Hospital, Benin City. All microbial isolates were confirmed using Gram staining and biochemical test as described in[23,24].

Preparation of Media and Biochemical Reagents

Nutrient agar, nutrient broth, sabouraud agar, sabouraud broth, Gram reagents and sugars for fermentation test were of analytical grade and prepared according to manufacturer instructions[23].

Sensitivity Test

A preliminary antimicrobial susceptibility test was carried out using the agar ditch method. Twenty milliliters of molten nutrient agar was poured into standard size Petri dishes and allowed to solidify, a ditch of 10mm wide and 60mm long was made in the center of the solidified nutrient

agar plate. The base of the ditch was sealed with molten agar to prevent the extract from sipping under the agar. Diluted over night nutrient broth culture of the all bacteria to match 0.5 Markfarland standard were streaked across the ditch, each ditch was 2/3 filled with 800 mg/ml of ethanol, aqueous extract and 750 µg/ml of ciprofloxacin. They were incubated right side up at 37^{o}C for 24hr. The same was done for *Candida albicans* using sabouraud agar. A lane of inhibition from the ditch indicates that the organism is sensitive.

Minimum Inhibitory Concentration

The minimum inhibitory concentration of the sensitive bacteria and fungi were determined using the agar well diffusion method. Twenty milliliters of molten nutrient agar were poured aseptically into standard Petri dishes and allowed to solidify. Six wells evenly spaced out were made in the nutrient agar and sabouraud agar with sterile 10 mm cork borer after seeding with the appropriate organisms. The base of each well was sealed with 0.025 ml of molten agar. The wells were labeled and were filled with 0.2 ml of the different concentrations of the extracts. The same procedure was used for Ciprofloxacin. The nutrient agar plates were incubated at 37^{o}C for 24 h and the sabouraud agar plates were incubated at room temperature for 72h.The growth pattern was observed and the zone of inhibitions were measured using a pair of divider and meter rule in millimeters.

RESULTS AND DESCUSSIONS

Preliminary Sensitivity

There was a lane of no growth between the edge of the ditch and where the growth was observed. The distance of lane away from the ditch shows the degree of sensitivity of the organism to the ethanol, aqueous extracts and Ciprofloxacin. The gram positive bacteria had longer lane compared to the gram negative. There was no lane of inhibition for *Escherichia coli* and *Pseudomonas aeruginosa* in the ditch with Ciprofloxacin.

Minimum Inhibitory Concentration

Ethanol Extract

The minimum inhibitory concentration (MIC) for the different organisms in the ethanol extract are; *Escherichia coli* ATCC 25922 (140 mg/ml), *Escherichia coli* (120 mg/ml), *Pseudomonas aeruginosa* ATCC 10145, (6 mg/ml), *Pseudomonas aeruginosa* (20 mg/ml), *Bacillus subtilis* NCTC 8236(4 mg/ml), *Bacillus subtilis* (8 mg/ml), *Staphylococcus aureus* ATCC (4 mg/ml) *Staphylococcus aureus* (1 mg/ml), *Candida albicans* ATCC 24433(120 mg/ml),*Candida albicans* (6 mg/ml) and *Klebsiella spp.*(200 mg/ml). The zones of inhibition decreased with decreasing concentration as depicted in Table 1.

Aqueous Extract

Minimum Inhibitory Concentration: Table 2, reports that the zones of inhibition of organisms decreased with decrease in concentration; *Escherichia coli* ATCC 25922(160 mg/ml); *Escherichia coli* (20mg/ml), *Pseudomonas aeruginosa* ATCC 10145, (20 mg/ml); *Pseudomonas aeruginosa* (40mg/ml), *Bacillus subtilis* NCTC 8236(20 mg/ml),*Bacillus subtilis* (40mg/ml), *Staphylococcus aureus* ATCC 25923(8 mg/ml),*Staphylococcus aureus* (8 mg/ml)*Candida albicans* (100mg/ml) and *Klebsiella spp*. (200 mg/ml). The aqueous extract had no activity against *Candida albicans* ATCC 24433. The MIC of the typed cultures was lower than those of the clinical isolates except *Staphylococcus aureus* which had the same MICs (8mg/ml).

Ciprofloxacin

Table 3, shows the results of antibacterial activity (MIC) of ciprofloxacin against the tested bacteria. *Escherichia coli* and *Pseudomonas aeruginosa* were resistant to ciprofloxacin. The zones of inhibition decreased with decreasing concentration as also observed in ethanol and aqueous extracts. *Escherichia coli* ATCC 25922 had (3.75 µg/ml), *Pseudomonas aeruginosa* ATCC 10145(0.23 µg/ml), *Bacillus subtilis* NCTC 8236(0.94 µg/ml), *Bacillus subtilis* (0.94 µg/ml), *Staphylococcus aureus* ATCC2523(1.86 µg/ml)*Staphylococcus aureus*(3.75 µg/ml) and *Klebsiella spp.*(0.23 µg/ml).

Table 1: *ANOGEISSUS LEIOCARPUS* ETHANOL EXTRACT ZONES OF INHIBITION (mm) AND MINIMUN INHIBITORY

Organism	800	600	400	200	180	160	140	120	100	80	60	40	20	10	8	6	4	2	1	0.8	0.6	0.4	0.2	MIC (mg/ml)
Escherichiacoli ATCC 25922	25	22	19	16	15	14	13	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	140
Escherichia coli	28	23	20	17	16	15	14	13	12	G	G	G	G	G	G	G	G	G	G	G	G	G	G	120
Pseudomonas aeuginosa 10145	35	32	29	26	23	22	21	20	19	18	17	16	15	14	13.5	13	G	G	G	G	G	G	G	6
Pseudomonas aeruginosa	33	30	27	24	23	22	21	20	19	18	17	16	15	G	G	G	G	G	G	G	G	G	G	20
Bacillus subtilis NCTC 8236	34	31	28	25	24	23	22	21	20	19	18	17	16	14.5	14	13.5	13	G	G	G	G	G	G	4
Bacillus subtilis	33	30	27	24	23	22	21	20	19	18	17	16	15	13.5	13	G	G	G	G	G	G	G	G	8
Staphylococcus aureus	38	35	32	29	28	27	26	25	24	23	22	21	20	19	18.5	18	17.5	17	17	G	G	G	G	1
Staphylococcus aureus ATCC 25923	39	37	34	30	29	28	27	26	25	24	23	22	21	20.5	20	19.5	19	G	G	G	G	G	G	4
Candidaalbicans ATCC 24433	28	25	22	19	18	17	16	15	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	120
Candida albicans	37	34	31	28	27	26	25	24	23	22	21	20	19	18	17.5	17	G	G	G	G	G	G	G	6
Klebsiellaspp	25	22	19	16	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	200

KEY: G=Growth.

Table 2: ANOGEISSUS LEIOCARPUS AQUEOUS EXTRACT ZONES OF INHIBITION (mm) AND MINIMUM INHIBITORY CONCENTRATION (mg/ml).

Organism	800	600	400	200	180	160	140	120	100	80	60	40	20	10	8	6	4	2	MIC (mg/dl)
Escherichia coli ATCC 25922	21	19	17	15	14	13	G	G	G	G	G	G	G	G	G	G	G	G	160
Escherichia coli	19	17	15	13	G	G	G	G	G	G	G	G	G	G	G	G	G	G	200
Pseudomonas aeruginosa ATCC 10145	28	26	24	22	21	20	19	18	17	16	15	14	13	G	G	G	G	G	20
Pseudomonas aeruginosa	27	25	23	21	20	19	18	17	16	15	14	13	G	G	G	G	G	G	40
Bacillus subtilis NCTC 8236	29	27	25	22	21	20	19	18	17	16	15	14	13	G	G	G	G	G	20
Bacillus subtilis	27	25	23	21	20	19	18	17	16	15	14	13	G	G	G	G	G	G	40
Staphylococcus aureus ATCC 25923	33	31	29	27	26	25	24	23	21	20	19	18	17	16.5	16	G	G	G	8
Staphylococcus aureus	30	28	26	24	23	22	21	20.5	20	19	18	17.5	17	16.5	16	G	G	G	8
Candida albicans ATCC 24433	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	-
Candida albicans	24	23	21	19	18	17	16	15	14	G	G	G	G	G	G	G	G	G	100
Klebsiella spp.	20	18	16	14	G	G	G	G	G	G	G	G	G	G	G	G	G	G	200

KEY: G—Growth.

Table 3: CIROFLOXACIN ZONES OF INHIBITION (MM) AND MINIMUM INHIBITORY CONCENTRATIONS (µg/ml)

Organism	750	650	550	450	250	240	120	60	30	15	7.5	3.75	1.86	0.94	0.47	0.23	0.12	0.06	0.03	0.01	MIC(ug/ml)
Escherichia coli ATCC 259 22						36	34	32	29	26	23	20	G	G	G	G	G	G	G	G	3.75
Escherichia coli	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	-
Pseudomenas aeruginosa 10145						34	32	30	28	26	24	23	21	19	17	15	G	G	G	G	0.23
Pseudomonasl aeruginosa	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	-
Bacillus subtilis NCTC 8236						33	31	29	27	25	23	20	17	14	G	G	G	G	G	G	0.94
Bacillus subtilis						34	32	30	28	26	24	21	18	15	G	G	G	G	G	G	0.94
Staphylococcus aureus ATCC 25923						38	36	34	31	28	25	22	19	G	G	G	G	G	G	G	1.86
Staphylococcus aureus						41	38	34	30	26	22	18	G	G	G	G	G	G	G	G	3.75
Klebsiella spp.						39	37	35	33	31	29	27	25	22	19	16	G	G	G	G	0.23

KEY: G—Growth.

DISCUSSION

Anogeissus leiocarpus plant parts have been used by Herbal medicine practitioners for the treatment of microbial infections without the scientific knowledge of antimicrobial activity. This study confirmed why the treatment was successful as both the ethanol and aqueous extracts had antimicrobial activities against all the tested organisms except *Candida albicans* ATCC 24433 that was not inhibited by the aqueous extract, this finding collaborates with that of[25]. The zones of inhibition produced indicate the susceptibility of the tested organisms to the extracts and the zones of inhibition decreased with decreasing concentrations of both ethanol and aqueous extracts, this result agrees with the scientific report of [26,27,12]. The ethanol extract was more potent than the aqueous extract, using a two-way anova without replication $P < 0.05$ for the zones of inhibition and $P < 0.05$ for the minimum inhibitory concentration (MIC), the ethanol plant extract had lower minimum inhibitory concentration which shows that this extract had more substances with antimicrobial properties. [28,29] had reported that ethanol is known to extract more secondary plant metabolites with antimicrobial activity on the tested isolates however a wealth of water soluble substances with antimicrobial activity were extracted from the leaves of *Anogeissus leiocarpus* which justify the use of the plant decoction in the treatment of diseases by traditional medicine practitioner was reported by[25]. There was no significant difference between the zones of inhibition of the standard organisms (typed cultures) and the clinical isolates in both the ethanol and aqueous extracts. In ethanol extract, the MIC of typed *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Candida albican* ATCC 24433 were higher than those of the clinical isolates while *Pseudomonas aeruginosa* ATCC 10145 and *Bacillus subtilis* NCTC8236 had lower MICs compared to the clinical isolates but in aqueous extract the MICs of typed *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 10145 and *Bacillus subtilis* NCTC 8236 were lower than those of clinical isolates, *Candida albicans* ATCC 24433 had no MIC as it was not inhibited. Typed and clinical isolates of *Staphylococcus aureus* had the same MIC of 8 mg/ml. The results of the standard drug ciprofloxacin had lower MICs compared to the aqueous and ethanol extract however it was not able to inhibit *Escherichia coli* and *Pseudomonas aeruginosa* that were inhibited by the plant root extracts. The plant root extracts had higher MICs because the extracts were used in the crude form and the active substance was not isolated and tested in this work. The plant extracts could be said to have a wider antibacterial activity as they inhibited *Escherichia coli* and *Pseudomonas aeruginosa* that were resistant to ciprofloxacin.

CONCLUSION

The result of this study revealed the high antimicrobial substances possessed by the *Anogeissusleiocarpus* root. The plant extracts were able to inhibit all the tested standard and clinical organisms except *Candida albicans* ATCC 24433 in aqueous extract which was resistant. The crude ethanol extract had MIC as low as 1.0mg/ml for clinical *Staphylococcus aureus*. Some bacteria that were not susceptible to ciprofloxacin were inhibited by the plant root extracts which shows that the plant had wider antibacterial activity. It is therefore recommended that work to be done to isolate the active antimicrobial constituent(s) of the plant and its toxicity level determined.

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