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Antimicrobial activity of the roots and stem bark of *Ceiba pentandra* and *Anogeissus leiocarpus* grown in Bauchi, North Eastern Nigeria

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Abstract
The roots and steam barks of *Ceiba pentandra* and *Anogeissus leiocarpus* was prepared by extracting 100_g of the crude sample using the hot soxhlet extraction with n-hexane, ethyl acetate, acetone, methanol and water sequentially in order of their polarity index. The extracts were tested against some clinical isolates of *Eschericia coli*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*. The results showed that the water stem bark extracts of *Anogeissus leiocarpus* W(S) B showed the highest activity with 20.00ug/ml, while the Acetone roots of *Anogeissus leiocarpus* extracts A(R)B had the least activity with 0.00mg/ml. in case of the antibacterial activity while in the case of the antifungal activity the water stem extracts of *Ceiba pentandra* had the highest activity with 15.00ug/ml, while the Acetone roots of *Anogeissus leiocarpus*, methanol roots of extracts of *Ceiba pentandra* A(R)B, M(R)A and W(R)A had the least activity of 0.00, 0.00, and 0.00 ug/ml antifungal activity. Streptomycin was used as the standard for the antibacterial activity while Fulcin was used as the standard for the antifungal activity. The antimicrobial activity of the roots and stem barks of *Ceiba pentandra* and *Anogeissus leiocarpus* justifies the claims by the traditional healers that the roots and stem barks of the plants in used to cure different ailments.

Keywords: *Ceiba, pentandra,* Streptomycin, Polarity, *Anogeissus* and Fulcin

Introduction

The use of plants as medicine to cure illness or diseases and to lubricate the wheel of social interaction at interpersonal- level is a behavior that predicts civilization. It is found in every society irrespective of its level of development and sophistication (Odugbemi, 2006) [7]. Medicinal plants contains numerous biologically active compound such as nutrient and phytochemicals which have physiological actions on the human body (Edeoga *et al*., 2005) [5].

Mathew (1996) observed that 70% to 80% of the populace in Nigeria rely on plants for their primary health care needs. Today phytochemists and pharmaceutical companies depends on those medicinal plants. The potential of the higher plants as a sources of new drugs is still largely unexpressed as among the estimated 250,000 – 500,000 plants species, only a small percentage has been investigated for their phytochemical constituents and the fractions subjected to biological or pharmacological screening (Makes and Satish, 2008).

The success story of chemotherapy can only lie in one continuous search for new drugs to counter the challenges posed by resistant strain of microorganisms (Doughari *et al.*, 2008) [10].

Studies have confirmed that extracts from *Ceiba pentandra* and *Anogeissus leiocerpus* as well as their isolated compound possess the diverse biological activities including anti-inflammatory, antitumor, antimicrobial, antioxidant, anti-ulcer to protective with alkaloid and flavones as the major active compound.

The juice obtained from the roots is applied typically for the treatment of skin infectious. In Africa stem bark extracts of *Ceiba pentandra* and *Anogeissus leiocarpus* are used to treat leprous infections, gonorrhea, syphilis and sores (Burkill, 2000). In Brazil the roots extracts are used as antipyretic and anti-inflammator (Moreira, 2010). In Nigeria, the juice from both the roots and stem barks are used for the treatment of diabetes I and II and also for the Hepatitis (Sofowora, 1993) [11].

The specific objectives of the study includes extracting and isolating the active ingredient from both the roots and stem barks, to confirm or disprove the efficacy of the plants by evaluating their antibacterial and antifungal activity.

Materials and Methods

Collection of the sample

Ceiba pentandra and *Anogeissus leicocarpus* roots and stem barks were collected from their natural habitat of the coastal plain of Bayara a suburb of Bauchi town in the months of October, 2015. The samples were air dried for about two (2) weeks under shade and milled in to fine powder using Wiley milling machine. 100g of the pulverized sample of the extracts were weighed and extracted using the hot solvent extraction with n-hexane, ethyl acetate, acetone, methanol and distilled water sequentially in order of their polarity index. The extracts were stored in a dissecator for the use.

Microorganisms

The micro-organisms for the test includes *Eschericia coli*, *staphylococcus aureus*, *Aspergillus niger* and *Candida albicans*. They were obtained from the reference organisms of Microbiology unit of Abubaka Tafawa Balewa University Bauchi. The choice of these pathogens was based on their implications in human diseases such as Typhoid fever, Pneumonia, Urinary infections, diabetes, hepatitis etc.

Antimicrobial Test

The antimicrobial activity was carried out on the crude extracts against the microorganisms. It was used as a guide to determine the active components of the roots and stem barks of *Ceiba pentandra* and *Anogeissus leicarpus*. The procedures of water birth (1978) and Perez *et al*. (1990) [9] were employed with small modifications. The methods involved the preparation of the culture medium and inoculations. Aseptic technique was used to avoid contamination.

Preparation of the Media

Two media were employed for this research, Nutrient Agar (NA) for bacteria culture and Malt extract agar (MEA) for fungi culture. The media was prepared by dissolving 14g of NA in 1000cm^3 of distilled water while 25g of MEA was dissolved in 1000cm^{3}of distilled water. They were sterilized at 121^{0}C for 15 minutes in an auto clave and subsequently allowed to cool to about 45^{0}C (temperature at which the agars remains molten) and formed in a plate (petri dish) and allowed to gel or solidified.

Standardization of Inoculums

The four test organisms were sub cultured with nutrient both using both a wire loop (aseptically) and incubated for 24h r at 35^{0}C for bacterial and 48h at 25^{0}C for fungi. The growth of the micro-organisms in the broths by turbidity produced was adjusted to march 0.lmc farland standard (10^{9}cfu/mc) which was further changed to 10^6 cfu/ml for both the pathogens.

Application of the Extracts and Innoculation of the Plates

The Agar plates NA and MEA were inoculated by spreading a small volume of (0.04 to 0.10ml) of the liquid inoculums (Sub cultured nutrient broth) by a means of glass root. One microbe was inoculated to each plates making a total of six (6) plates for each microbes. Six (6) wells for the acetone, methanol and water extracts and two (2) for the control (streptomycin for bacteria and fulcin for fungi). The inoculated plates were left for several hours in order to allow the extracts to diffuse into the agar. The nutrient agar and malt extract agar were incubated at 37^{0}C for 24 hours for bacteria and 36 hours for fungi. The diameter of the zone of inhibition were determined using the vernier caliper and it was recorded.

Results

Table 1: The zone inhibition (mm) of the antibacterial activity of the crude extracts of acetone roots and stem barks of *Ceiba pentendra* and *Anogeissus leiocarpus*.

Test	Con (mg/m)	A(r)a	A(r)b	A(s)a	A(s)b	Streptomycin
Staphyloccus Aureus	10^{-1}	10.00	8.00	12.00	14.00	0.00
	10^{-2}	10.00	6.00	10.00	12.00	0.00
	10^{-3}	8.00	4.00	8.00	10.00	0.00
	10^{-4}	6.00	2.00	6.00	8.00	0.00
Eschericia coli	10^{-1}	6.00	5.00	14.00	16.00	0.00
	10^{-2}	4.00	3.00	12.00	14.00	0.00
	10^{-3}	4.00	3.00	10.00	12.00	0.00
	10^{-4}	2.00	0.00	10.00	10.00	0.00

Keys: A(R)A – Acetone roots of *Ceiba pentandra*
A (R)B – Acetrone roots of *Anogeisus leiocarpus*
A (R)B – Acetone stem of *Ceiba Pentandra*
A (R)B – Acetone stem of *Anogeissus leiocarpus*
SM - Streptomycin (control).

Table 2: Zone of inhibition (mm) of the antibacterial activity of the crude extracts of methanolic extracts of the roots and stem barks of C*eiba pentandra* and *Anogeissus lieocarpus*

Test obtained	Con (mg/ml)	M(r)a	M(r)b	M(s)a	M(s)b	Streptomycin
Staphyloccus Aureus	10^{-1}	10.00	9.00	12.00	15.00	0.00
	10^{-2}	8.00	9.00	10.00	13.00	0.00
	10^{-3}	6.00	6.00	8.00	10.00	0.00
	10^{-4}	6.00	4.00	8.00	10.00	0.00
Eschericia coli	10^{-1}	11.00	11.00	13.00	16.00	0.00
	10^{-2}	10.00	9.00	11.00	14.00	0.00
	10^{-3}	10.00	6.00	8.00	12.00	0.00
	10^{-4}	8.00	4.00	6.00	8.00	0.00

M (R)A- Methanol roots of *Ceiba pentandra*
M (R)B -Methanol roots of *Anogeissus leiocarpus*
M (S)B- Methanol stem of *ceiba pentandra*
M (S)B - Methanol stem of *Anogeissus leiocarpus*
ST- Streptomycin (control).

Table 3: Zone of inhibition (mm). The antibacterial activity of the crude extract of water, extracts of water roots steam of *Ceiba pentandra* and *Anogeissus leiocarpus*

Test organisms	Con (mg/ml)	M(r)a	M(r)b	M(s)a	M(s)b	Streptomycin
Staphyloccus Aureus	10^{-1}	14.00	12.00	17.00	18.00	0.00
	10^{-2}	12.00	10.00	15.00	15.00	0.00
	10^{-3}	12.00	8.00	13.00	12.00	0.00
	10^{-4}	10.00	6.00	10.00	12.00	0.00
Eschericia coli	10^{-1}	11.00	10.00	18.00	20.00	0.00
	10^{-2}	10.00	9.00	18.00	18.00	0.00
	10^{-3}	8.00	6.00	16.00	16.00	0.00
	10^{-4}	6.00	4.00	12.00	14.00	0.00

W(R)A –Water roots of *ceiba pentandra*
W(R)B -Water roots of *Anogeissus leicarpus*
W(S)A- Water stem of *ceiba pentandra*
W(S)A- Water stem of *Anogeissus leiocarpus*
ST – Streptomycin (control)

Table 4: Zone of inhibition (mm) of antifungal activity of the crude extracts of the Acetone roots and stem barks of *Ceiba pentandra* and *Anogeissus leiocarpus*.

Test organisms	Con (mg/ml)	A(r)a	A(r)b	A(s)a	A(s)b	FL
Candida albicans	10^{-1}	6.00	7.00	10.00	12.00	0.00
	10^{-2}	2.00	5.00	8.00	10.00	0.00
	10^{-3}	0.00	3.00	6.00	6.00	0.00
	10^{-4}	4.00	2.00	4.00	4.00	0.00
Aspergillus niger	10^{-1}	4.00	5.00	8.00	9.00	0.00
	10^{-2}	2.00	3.00	6.00	7.00	0.00
	10^{-3}	0.00	2.00	4.00	5.00	0.00
	10^{-4}	0.00	0.00	2.00	2.00	0.00

A(R) A -Acetone roots of *Ceiba pentandra*
A(R) B -Acetone roots of *Anogeissus leiocarpus*
A(S) A- Acetone stem of *Ceiba pentandra*
A(S) B- Acetone stem of *Anogeissus leiocarpus*
FL– Fulcin (control)

Table 5: Zone of inhibition (mm) of antifungal activity of the crude extracts of the methanol roots and stem barks of *Ceiba Pentandra* and *Anogeissus leiocarpus*.

test organisms	con (mg/ml)	a(r)a	a(r)b	a(s)a	a(s)b	fl
Candida albicans	10^{-1}	6.00	7.00	10.00	12.00	0.00
	10^{-2}	4.00	5.00	8.00	10.00	0.00
	10^{-3}	2.00	3.00	6.00	6.00	0.00
	10^{-4}	0.00	2.00	4.00	4.00	0.00
Aspergillus niger	10^{-1}	4.00	5.00	8.00	9.00	0.00
	10^{-2}	2.00	3.00	6.00	7.00	0.00
	10^{-3}	0.00	2.00	4.00	5.00	0.00
	10^{-4}	0.00	0.00	2.00	2.00	0.00

Keys: A(R)A –Acetone roots of *Ceiba Pentandra*
A(R)B –Acetone roots of *Ceiba Pentandra*
A(S)A- Acetone stem of *Anogeissus leiocarpus*
A(S)B- Acetone stem of *ceiba pentandra*
FL – Fulcin (control).

Table 6: Zone of inhibition (mm) of antifungal activity of the crude extracts of the methanol roots and stem barks of *Ceiba pentandra* and *Anogeissus leiocarpus.*

test organisms	con (mg/ml)	w(r)a	w(r)b	w(s)a	w(s)b	FL
Candida Albicans	10^{-1}	14.00	12.00	15.00	13.00	0.00
	10^{-2}	12.00	10.00	12.00	10.00	0.00
	10^{-3}	10.00	8.00	10.00	8.00	0.00
	10^{-4}	8.00	6.00	8.00	6.00	0.00
Aspergillus niger	10^{-1}	8.00	10.00	10.00	11.00	0.00
	10^{-2}	6.00	8.00	8.00	8.00	0.00
	10^{-3}	4.00	6.00	6.00	6.00	0.00
	10^{-4}	0.00	4.00	6.00	4.00	0.00

Keys: W(R)A –Water roots of *ceiba pentandra*
W(R)B –Water roots of *Anogeissus leicacarpus*
W(S)A- Water stem of *ceiba pentandra*
W(S)B- Water stem of *Anogeissus leicacarpus*
FL – Fulcin (control).

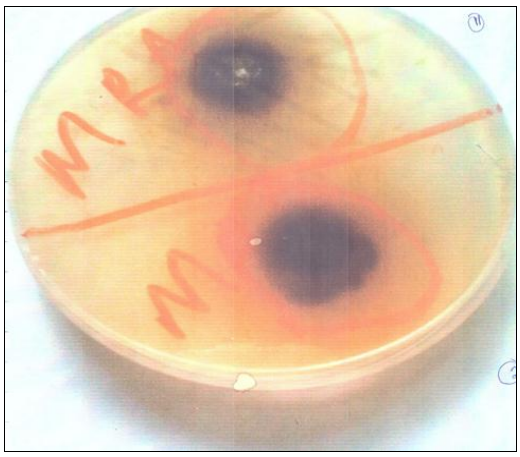

Fig 1: The zone of Inhibition of M(R) A and M(R)B on *Escherica coli*

Fig 2: The zone of Inhibition of A(R) A and A(R)B on *Candida albicans*

Fig 3: The zone of Inhibition of A(R)A and A(R)B on *Eschericia coli*

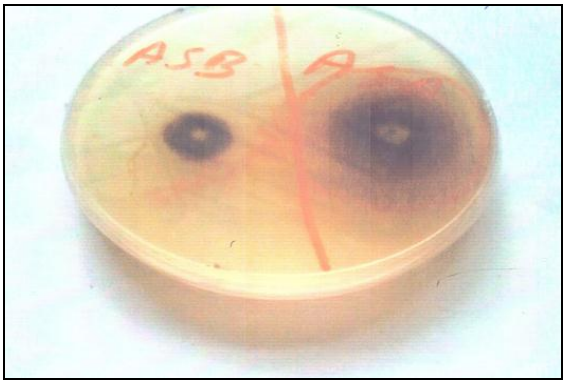

Fig 4: The zone of Inhibition of A(S)B and A(S)A on *Candida albicans*

Fig 5: The zone of Inhibition of A(S) B and A(S)A on *Eshericia coli*

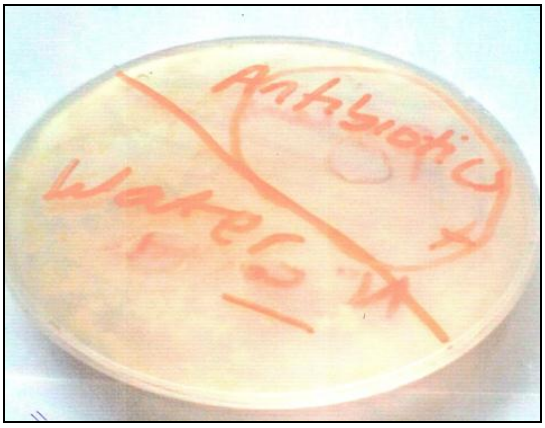

Fig 6: The zone of Inhibition of Antibiotic (streptomycin) used as the positive control and water as the negative control

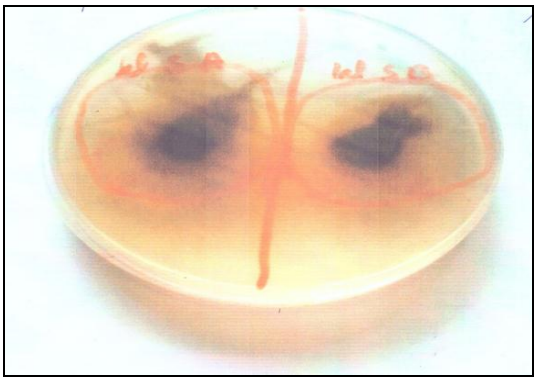

Fig 7: The zone of Inhibition of W(S)A and W(S)B on *Candida albicans*

Fig 8: The zone of Inhibition of W(R) A and W(R)B on *Candida albicans*

Fig 9: The zone of Inhibition of M(R)A and M(R)B on *Candida albicans*

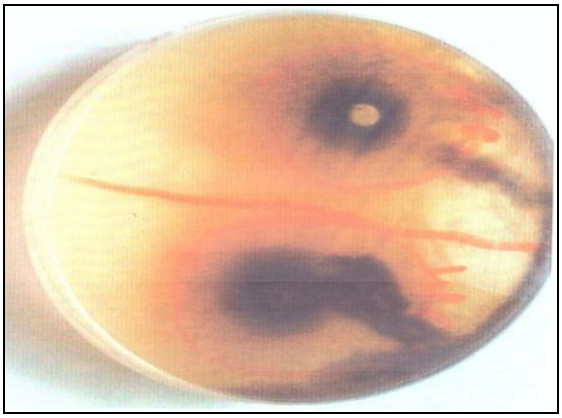

Fig 10: The zone of Inhibition of W(R) A and W(R) B on *Escherichia coli*

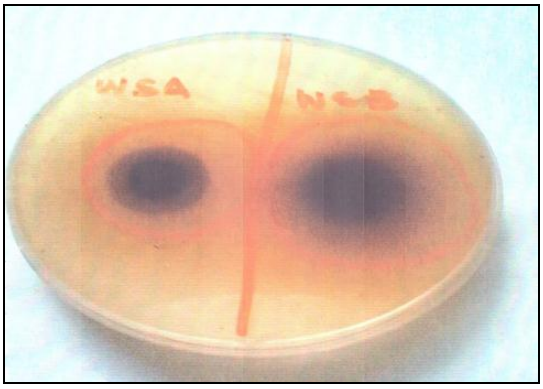

Fig 11: The zone of Inhibition of W(S) A and W(S) B on *Escherichia coli*

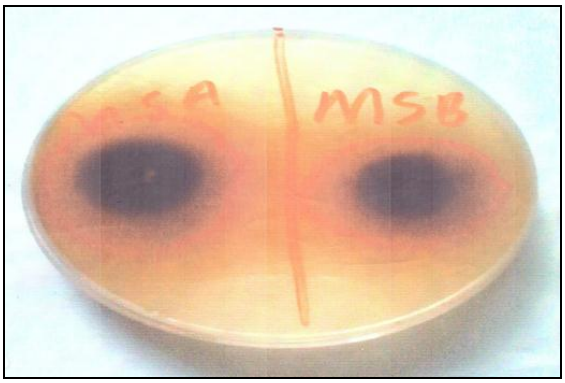

Fig 12: The zone of Inhibition of M(S)A and M(S)B on *Eschericia coli*

Discussion

Antimicrobial activity of the roots and stem barks of *Ceiba pentandra* and *Anogeissus leiocarpus* was carried out using the clinical isolate of *staphylococcus aureus, Eschericia coli, Candida albicans* and *Aspergillus niger*.

Monica (1984) [6] pointed out that the active antimicrobial compound diffuses from the disc into the medium and the organism are sensitive to the active antimicrobial compound are inhibited at distance from the disc. The antimicrobial activity of the roots and stem barks of *Ceiba pentandra* and *Anogeissus leiocarpus* was against the growth of the microorganisms (microbes) were observed visually and measured. Adewumi and Sofowora (1980) pointed out that the roots and extracts of *Ceiba pentandra* possess antimicrobial actions at different concentration depending on the bacterial species. The measured zone of inhibition of the pathogens by the crude extracts are summarized in (Table 1 – 6) and (Figure 1 – 11).

The antibacterial and antifungal assays were performed for acetone, methanol and water fractions of the roots and stem barks of *Ceiba pentandra* and *Anogeissus leiocarpus* respectively against the chemical isolates of *staphylococcus aurens, Escherice coli, Aspergillus niger* and *candida albicans*.

Table 1 shows the result resists of the zone inhibition of the extracts on the clinical isolates. This proves that extracts were active at appropriate concentration against the microbes with the increase in the zone of inhibition tends to reduces as in the case of acetone extracts (Table 1).

The invitro antimicrobial activities results obtained from the roots and stem barks of *Ceiba pentandra* exhibited antibacterial activities (Table 1 – 3) with the exceptions of the *C. abicans* and *A. niger* which had the least effects with the decrease in the concentration (Table IV).

Methanol and water extracts of the roots and stem of *Ceiba pentandra* and *Anogeissus leiocarpus* exhibited a significant antibacterial activities on the *S. aureus* and *E. cole* (Table II and III), while showed the least anti-fungal activities (Table V and VI).

The dimension of the zone of inhibition obtained for all the extracts all obtained of the (Figure 1 – 11).

Abad *et al.* (2009) [2] Have listed *saponins Terpenoids, Flavonoids, phenolics* and steroidal compounds, are among the phytochemicals with antimicrobial effects and there phyto compounds are present in all the extracts.

The results showed increase in the antibacterial activity with increase in polarity of the solvents used in the extraction. They could mean that the concentrations of the phytochemical are higher in the polar solvent extract than the least (polar extracts). It is also noted that phytoconpounds are not presents or they are less in the acetone fractions (Table IV). It is known that compound of plants origin do not have the same antimicrobial and antifungal effects on individual organisms while some could be highly antibacterial some could be highly antifungal.

Conclusion

This justifies the claims by the traditional healers that the roots and stem barks of *Ceiba pentandra* and *Anogeissus leiocarpus* are used to cure illness has been confirmed due to their antipathogenic activity and could be used for the synthesis of new drugs in pharmaceutical companies.

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