

**COMPARATIVE STUDY ON ANTIMICROBIAL AND PHYTOCHEMICAL
PROPERTIES OF DIFFERENT POLAR FRACTIONS OF
Anogeissus leiocarpus ROOT EXTRACT FROM LANGTANG LGA, PLATEAU
STATE.**

Comment [PM1]: What is the gap of this study?
Methodology of each objective is not clear

Abstract:

Anogeissus leiocarpus plant is widely used in Africa and among Tarok people in the northern senatorial zone of Plateau State, Nigeria as antimicrobial agents against many pathogenic microorganisms. This study was carried out *in vitro* to compare the antibacterial and antifungal properties of non-polar (hexane, ethyl acetate) and polar (methanol, water) root extracts against clinical isolates of *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhi*, *Escherichia coli*, *Aspergillus flavus*, *Trichophyton rubrum*, *Aspergillus brasiliensis*, and *Candida albicans*. The extracts of polar solvents showed strong antibacterial activity against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhi*, *Escherichia coli*, and *Candida albicans*. But for *Aspergillus flavus*, and *Trichophyton rubrum* both the polar and non-polar solvents showed equal inhibition. Ciprofloxacin, Fluconazole and Amphotericin B were used as control. The result revealed that the selected micro-organisms were sensitive within the concentration range of 50 – 400mg/ml. Preliminary phytochemical results revealed the presence of important bioactive substances such as cardiac glycosides, tannins, saponins, steroids, carbohydrates, flavonoids and terpenes. Thus, methanol extracts contain bioactive compounds that could be utilized in developing new antibiotics.

Keywords: *Anogeissus leiocarpus*, clinical isolates, antibacterial activity, bioactive, Polar fractions.

INTRODUCTION:

In Africa, our forefathers were known for using plants for the treatment of various diseases. One of such plants is *Anogeissus leiocarpus* popularly known as marke in Hausa ^[1]. Both the Sudanese and Taroke people of northern senatorial of Plateau state use this plant for traditional medicine and is well known antimicrobial activities against many pathogenic micro-organisms for treating diseases ^[2]. This diseases includes; toothache, diarrhea, respiratory diseases, jaundice, hepatitis, haemorrhoids, headache and as

antimalarial, leprotic, laxative and anthelmintic^[3] skin diseases and infections, wounds infections, sore feet, boils, cysts, syphilitic and diabetic ulcers^[4]. It showed strong antibacterial and antifungal activity against many pathogenic micro-organisms^[5].

Anogeisussleiocarpus belong to the family of combretaceae which according to research contain high concentrations of flavonoids, terpenoids, tannins or polyphenolic compounds, which were known for their antimicrobial activity^[6]. Other compounds includes ellagitannins and stilbenes^[7]. The genus of *Anogeisuss* also contain the following metabolite with antimicrobial activities; Tannins, polyphenol, flavonoids, steroids, stilbenes and lignan^[8].

The purpose of this research is to make a comparison of the active compounds in different polar and non-polar fractions of root extracts that are responsible for microbial inhibition of eight micro-organisms (*Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhi*, *Escherichia coli*, *Aspergillus flavus*, *Trichophyton rubrum*, and *Candida albicans*) which are responsible for many disease such as toothache, diarrhea, respiratory diseases, and skin diseases and infections^[9,10].

EXPERIMENTAL:

Materials:

The following materials were used for the research;

(i) Hexane, ethyl acetate, methanol of ASTM grade of 99.85 % and water. (ii) Nutrient agar (iii) Ciprofloxacin, Amphotericin and Fluconazole.

Sample collection and preparation:

The roots sample of *Anogeisussleiocarpus* was collected from Lantang L.G.A in southern senatorial zone of Plateau State, Nigeria. And was taken to the Federal College of Forestry along Bauchi road opposite University of Jos, Jos Plateau State for identification by Mr. Joseph J. Azila. It was then washed under running water and dried in an oven for 72 hours under room temperature and grounded and sieved using 30 mm mesh size screen.

Successive Extraction was carried out starting with non-polar solvents (Hexane and Ethyl acetate) and then polar solvents (methanol and water) of 500 g of the dried root powdered sample for 6 h. The samples were then packaged for further analysis.

Comment [PM2]: The material

Comment [PM3]:

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Comment [PM5]: The material should specific such as brand, grade, country serial no

Comment [PM6]: What oshetrbarium no

Comment [PM7]: The volume for each solvent and yield



Fig. 1: *Anogeissus leiocarpus* tree.

Sample analysis:

(a) Microorganisms

The antimicrobial activity of the plant extract was evaluated using four bacterial isolates (*Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhi* and *Escherichia coli*) and three fungal isolates (*Aspergillus flavus*, *Trichophyton rubrum*, *Aspergillus brasiliensis*). The microorganisms were provided from the culture collection of Microbiology Section of Central Diagnostic Laboratory, National Veterinary Research Institute, Vom, Plateau State.

Comment [PM8]: The identification no or ATCC NO ?

(b) Standardization of inoculum

Pure culture of each organism was selected. Sterile wire loop was used to pick 2 to 3 colonies of the organism and sub cultured into 10 mL of nutrient broth (Oxoid, UK) and Mycological broth (Oxoid, UK) for bacteria and fungi respectively. The broths were incubated at 37 °C for 18 h and at 25 °C for 3 days. Fifty microliter (50 µl) was dispensed in a tube containing 5 ml of physiological saline. The tube was inserted into a sensititre nephelometer (TREK Diagnostic system, UK) after calibration, adjustment was made with extra diluents, where necessary. It was adjusted to match 0.5 McFarland standard (10^8 cfu/ml) and 10^3 cfu/ml^[8].

Comment [PM9]: How to prepare cfufr fungus?

(c) Bacterial susceptibility testing

Agar diffusion method was carried out as described by Mueller-Hinton agar (MHA)^[8],^{9]} and Sabouraud dextrose agar plates were prepared according to the manufacturer's instruction. They were incubated for sterility check at 37 °C and 25 °C for 24 h. the plates

were flooded with one thousand microliter (1000 μ l) of the standardized organism separately. Excess was drained off and allowed to remain on the bench for 10 minutes. A sterile cork borer of 5 mm diameter was used to make 5 wells on each plate. One hundred microliter (100 μ l) of the various extract concentrations (400, 200, 100 and 50 mg/ml) were dispensed into each well and into the remaining well, Ciprofloxacin (20 mg/ml) and Amphotericin B (20 mg/ml) were dispensed as positive control. The inoculated plates were left on the bench for 10 minutes to allow the extract to diffuse into the agar. The plates were incubated aerobically at 37 ° C for 24 h for bacteria and 25 ° C for 4 days for fungi. The diameter of zones of inhibition were measured using a meter rule and considered as indication for antimicrobial activity^[11].

(d) Determination of minimum inhibitory concentration (MIC)

Modified broth dilution method as described by^[11, 12] were used. Two fold serial dilution of the extract concentrations were prepared. Twenty microliter (20 μ l) of each bacterial inoculum was dispensed into each concentration. The tubes were incubated at 37 ° C and 25 ° C for 24 h. and 3 days for bacteria and fungi respectively. The MIC was considered as the lowest concentration which inhibited the growth of the respective organism^[11].

(e) Determination of minimum bactericidal/Fungicidal concentration (MBC/MFC)

The MBC was determined by sub culturing the lowest concentration of the extract exhibiting invisible growth (from inhibition growth of MIC) onto sterile MHA and SDA plates. The cultured plates were incubated at 37 ° C and 25 ° C for 24 h. and 3 days for bacteria and fungi respectively. The lowest concentration that yielded no single bacterial colony on the medium was taken as MBC and MFC^[11,12].

RESULTS:

Table 1:Photochemical^[13, 15] .

CONSTITUENTS	HEXANE	ETHYL ACETATE	METHANOL	WATER
Alkaloids	-	-	-	-
Saponins	-	+	+	+++
Taninns	-	-	++	+++
Flavonoids	-	+	+++	+++
Carbohydrate	-	-	++	+
Steroids	+++	++	+	-
Terpenes	-	-	-	-
Anthraquinones	+	-	-	-
Cardiac glycosides	+	++	++	-
600 g % Yield	0.40	0.80	10.33	6.18

KEY: + present

++ Average

+++ Very present

Table 2: ANTIBACTERIAL ACTIVITY

ORGANISM	CONCENTRATION OF EXTRACT (mg/ml)/AVERAGE DIAMETER OF ZONES OF INHIBITION (mm)				Extract	Positive control
	400	200	100	50		
						Ciprofloxacin (20 mg/ml)
<i>Staphylococcus aureus</i>	30	26	22	18	R. MeOH	22
<i>Staphylococcus aureus</i>	6	6	6	6	R. Hex	22
<i>Staphylococcus aureus</i>	20	18	14	10	R. H₂O	22
<i>Staphylococcus aureus</i>	12	10	6	6	R. EA	22

<i>Klebsiella pneumonia</i>	16	12	6	6	R. MeOH	28
<i>Klebsiella pneumonia</i>	6	6	6	6	R.Hex	28
<i>Klebsiella pneumonia</i>	22	20	14	8	R. H₂O	28
<i>Klebsiella pneumonia</i>	6	6	6	6	R. EA	28
<i>Salmonella typhi</i>	28	23	19	12	R. MeOH	24
<i>Salmonella typhi</i>	6	6	6	6	R. Hex	24
<i>Salmonella typhi</i>	17	11	10	6	R. H₂O	24
<i>Salmonella typhi</i>	16	14	12	8	R. EA	24
<i>Escherichia coli</i>	21	18	8	6	R. MeOH	28
<i>Escherichia coli</i>	6	6	6	6	R. Hex	28
<i>Escherichia coli</i>	6	6	6	6	R. H₂O	28
<i>Escherichia coli</i>	6	6	6	6	R. EA	28
<i>Bacillus subtilis</i>	21	16	12	8	R. MeOH	32
<i>Bacillus subtilis</i>	6	6	6	6	R. Hex	32
<i>Bacillus subtilis</i>	6	6	6	6	R. H₂O	32
<i>Bacillus subtilis</i>	13	12	10	6	R. EA	32
						Amphotericin B (10 µg)

<i>Aspergillus flavus</i>	6	6	6	6	R. MeOH	20
<i>Aspergillus flavus</i>	6	6	6	6	R. Hex	20
<i>Aspergillus flavus</i>	6	6	6	6	R. H₂O	20
<i>Aspergillus flavus</i>	6	6	6	6	R. EA	20
<i>Trichophyton rubrum</i>	6	6	6	6	R. MeOH	22
<i>Trichophyton rubrum</i>	6	6	6	6	R. Hex	22
<i>Trichophyton rubrum</i>	6	6	6	6	R. H₂O	22
<i>Trichophyton rubrum</i>	6	6	6	6	R. EA	22
						Fluconazole (20 mg)
<i>Candida albicans</i>	10	8	6	6	R. MeOH	18
<i>Candida albicans</i>	6	6	6	6	R. Hex	18
<i>Candida albicans</i>	6	6	6	6	R. H₂O	18
<i>Candida albicans</i>	6	6	6	6	R. EA	18

KEY: SA *Staphylococcus aureus*, KP *Klebsiella pneumonia*, ST *Salmonella typhi*, EC *Escherichia coli*, BS *Bacillus subtilis* AF *Aspergillus flavus*, TR *Trichophyton rubrum*, CA *Candida albicans*. 6 = 0 diameter.

R.H₂O root water extract, R.MeOH root methanol extract, R.Hex root hexane extract and R.EA root ethyl acetate extract.

Table 3: MINIMUM INHIBITORY CONCENTRATION (MIC)

ORGANISM	CONCENTRATION OF EXTRACT (mg/ml)							EXTRACT	MIC (mg/ml)
	400	200	100	50	25	12.5	6.25		
<i>Staphylococcus aureus</i>	-	-	-	-	-	+	+	R. MeOH	25
<i>Staphylococcus aureus</i>	-	-	- μ	+	μ	+	+	R. H ₂ O	100
<i>Staphylococcus aureus</i>	-	- μ	+	+	+	+	+	R. EA	200
<i>Staphylococcus aureus</i>					+				
<i>Klebsiella pneumonia</i>	-	- μ	+	+	+	+	+	R. MeOH	200
<i>Klebsiella pneumonia</i>	-	-	- μ	+	+	+	+	R. H ₂ O	100
<i>Salmonella typhi</i>	-	-	-	-	- μ	+	+	R. MeOH	25
<i>Salmonella typhi</i>	-	-	- μ	+	+	+	+	R. H ₂ O	100
<i>Salmonella typhi</i>	-	- μ	+	+	+	+	+	R. EA	
<i>Escherichia coli</i>	-	-	- μ	+	+	+	+	R. MeOH	100
<i>Bacillus subtilis</i>	-	- μ	+	+	+	+	+	R. MeOH	200

KEY: - no turbidity, + presence of turbidity, μ MIC.

Table 4: MINIMUM BACTERICIDAL CONCENTRATION (MBC)

ORGANISM	CONCENTRATION OF EXTRACT (mg/ml)							EXTRACT	MBC (mg/ml)
	400	200	100	50	25	12.5	6.2 5		
<i>Staphylococcus aureus</i>	-	-	- β	+	+	+	+	R. MeOH	100
<i>Staphylococcus aureus</i>	-	- β	+	+	+	+	+	R. H ₂ O	200
<i>Staphylococcus aureus</i>	- β	+	+	+	+	+	+	R. EA	400
<i>Klebsiella pneumonia</i>	- β	+	+	+	+	+	+	R. MeOH	400
<i>Klebsiella pneumonia</i>	+	+	+	+	+	+	+	R. H ₂ O	0
<i>Salmonella typhi</i>	-	- β	+	+	- μ	+	+	R. MeOH	200
<i>Salmonella typhi</i>	- β	+	+	+	+	+	+	R. H ₂ O	400
<i>Salmonella typhi</i>	-	- μ	+	+	+	+	+	R. EA	200
<i>Escherichia coli</i>	-	- β	+	+	+	+	+	R. MeOH	200

<i>Bacillus subtilis</i>	+	+	+	+	+	+	+		R. MeOH	0

KEY: β MBC, - no growth, + growth, β MBC.

Table 5: POSITIVE CONTROL - MINIMUM INHIBITORY CONCENTRATION (MIC)

	Ciprofloxacin (mg/ml)								
ORGANISM	20	10	5	2.5	1.25	0.625	0.3125	0.1562	MIC (mg/ml)
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	- μ	< 0.1562
<i>Klebsiella pneumonia</i>	-	-	-	-	-	- μ	+	+	0.625
<i>Salmonella typhi</i>	-	-	-	-	-	-	- μ	+	0.3125
<i>Escherichia coli</i>	-	-	-	-	-	-	- μ	+	0.3125
<i>Bacillus subtilis</i>	-	-	-	-	-	-	- μ	+	0.3125
	Amphotericin B (μg/ml)								
ORGANISM	10	5	2.5	1.25	0.625	0.3125	0.1562		MIC (μg/ml)
<i>Aspergillus flavus</i>	-	+	+	+	+	+	+		10
<i>Trichophyton rubrum,</i>	-	+	+	+	+	+	+		10
	Fluconazole (mg/ml)								
	20	10	5	2.5	1.25	0.625	0.3125	0.1562	MIC (mg/ml)
<i>Candida albicans.</i>	-	-	+	+	+	+	+	+	10

KEY: - = No turbidity, + =Turbidity, μ = MIC

Table 6: POSITIVE CONTROL - MINIMUM BACTERICIDAL/FUNGICIDAL CONCENTRATION (MBC/MFC)

		Ciprofloxacin (mg/ml)							
ORGANISM	20	10	5	2.5	1.25	0.625	0.3125	0.1562	MBC (mg/ml)
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	- μ	< 0.1562
<i>Klebsiella pneumonia</i>	-	-	-	-	-	- μ	+	+	0.625
<i>Salmonella typhi</i>	-	-	-	-	-	-	- μ	+	0.3125
<i>Escherichia coli</i>	-	-	-	-	-	-	- μ	+	0.3125
<i>Bacillus subtilis</i>	-	-	-	-	-	-	- μ	+	0.3125
		Amphotericin B (μ g/ml)							
ORGANISM	10	5	2.5	1.25	0.625	0.3125	0.1562		MFC (μ g/ml)
<i>Aspergillus flavus</i>	-	+	+	+	+	+	+		10
<i>Trichophyton rubrum</i>	-	+	+	+	+	+	+		10
		Fluconazole (mg/ml)							
	20	10	5	2.5	1.25	0.625	0.3125	0.1562	MFC (mg/ml)
<i>Candida albicans</i>	-	-	+	+	+	+	+	+	10

KEY: - = No turbidity, + =Turbidity, β = Minimum Fungicidal Concentration (MFC)/ Minimum Bactericidal Concentration (MBC).

DISCUSSION:

This study was carried out in vitro to compare the antibacterial and antifungal activities of non-polar (hexane, ethyl acetate) and polar (methanol, water) root extracts from Langtang against clinical isolates of *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhi*, *Escherichia coli*, *Aspergillus flavus*, *Trichophyton rubrum*, *Candida albicans*. The table 1, revealed much presence of bioactive compounds in polar extracts compare to

Comment [PM10]: The discussion should compare with the previous finding.. give the gap and why the result is different or same

extract of non-polar extracts. This suggests the reason for the strong antibacterial activity against *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhi*, *Escherichia coli*, and *Candida albicans*^[4]. The bioactive compounds in high quantity includes; Saponins, Taninns, Flavonoids and Carbohydrates. This result confirms the report by Ahmad H. A. et al (2014), that these bioactive compound can inhibit both bacteria and fungi^[10]. Table 2, showed that the polar extracts, particularly the methanol have strong activity against the bacteria isolates between 50 – 400 mg/ml. This could be due to the presence of much bioactive compounds as revealed by the phytochemical results. For the non-polar extracts, only the ethyl acetate extract gave moderate activity against *Staphylococcus aureus* and *Salmonella typhi* between 200 -400 mg/ml concentration. This could be as a result of the presence of moderate amount of Steroids and Cardiac glycosides^[10, 5]. Table 2 also showed that the polar extract had stronger activity against fungi compare to that of non – polar extracts. But there was no activity for *Aspergillus flavus* and *Trichophyton rubrum*, The presence of turbidity at a given concentration of the extracts is an indicator that there is inhibition by the extracts. Table 3 and 4 revealed that the methanol have minimum concentration at 25 and 100 mg/ml against *Staphylococcus aureus* for MIC and MBC analysis. The polar extract methanol showed no activity against the following fungi; *Aspergillus flavus*, *Trichophyton rubrum*, and *Candida albicans*. This investigation was done alongside with some standard drugs such as Ciprofloxacin, Amphotericin and Fluconazole. These drugs showed strong activity against all the microorganisms used for this investigation. Table 5 and 6 revealed that the MIC and MBC/MFC for Amphotericin and Fluconazole is 10 µg which greater compare to that of Ciprofloxacin within the range of < 0.1562 – 0.3125 mg.

Conclusion: The results from this research has revealed that due to the much presence of bioactive compounds such as Saponins, Taninns, Flavonoids, Carbohydrates, Steroids and Cardiac glycosides, the root extract of *Anogeissus leiocarpus* has the potential for treating diseases caused by microorganisms such *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhi*, *Escherichia coli*, *Aspergillus flavus*, *Trichophyton rubrum*, and *Candida albicans*. In addition, this work further affirms the claims by traditional healers of the potentials of the root of *Anogeissus leiocarpus* to heal diseases such as dental caries^[16], periodontal disease, respiratory tract infections, wound infection, diarrheal, respiratory tract inflammation, skin infection and candidiasis^[2, 14]. Finally, the root can be used as suitable replacement for synthetic orthodox drugs for bacteria treatment.

Comment [PM11]: How you classfied it is moderate

Comment [PM12]: What can be conclude from the result of MIC and MBC/MFC?

Comment [PM13]: It should answered the tittle.. how about the comparison of antimicrobial? Which one is good and why

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