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

Abstract:

Anogeissusleiocarpus 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 braziliensis,* 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 revelled 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: *Anogeissusleiocarpus*, 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 *Anogeisussleiocarpus 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 genious of Anogeisuss also contain the following metabolte with antimicrobial activities; Tannins, polyphenol, flavonoids, steroids, stilbenes and liginan[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 s toothache, diarrhea, respiratory diseases, and skin diseases and infections[9,10].

EXPERINMENTAL:

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.

Fig. 1: *Anogeisussleiocarpus*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.

(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 ^{o}C for 18 h and at 25 ^{o}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].

(c) **Bacterial susceptibility testing**

Agar diffusion method wad 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 at37 ^{o}C and 25 ^{o}C for 24 h. the plates

were flooded with one thousand microliter (1000 µl) 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 µl) 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 ^{o}C for 24 h for bacteria and 25 ^{o}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 µl) of each bacterial inoculum was dispensed into each concentration. The tubes were incubated at 37 ^{o}C and 25 ^{o}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 ^{o}C and 25 ^{o}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)**
Staphylococcus aureus	30	26	22	18	**R. MeOH**	22
Staphylococcus aureus	6	6	6	6	**R. Hex**	22
Staphylococcus aureus	20	18	14	10	**R. H_2O**	22
Staphylococcus aureus	12	10	6	6	**R. EA**	22

Klebsiella pneumonia	16	12	6	6	**R. MeOH**	28
Klebsiella pneumonia	6	6	6	6	**R.Hex**	28
Klebsiella pneumonia	22	20	14	8	**R. H_2O**	28
Klebsiella pneumonia	6	6	6	6	**R. EA**	28
Salmonella typhi	28	23	19	12	**R. MeOH**	24
Salmonella typhi	6	6	6	6	**R. Hex**	24
Salmonella typhi	17	11	10	6	**R. H_2O**	24
Salmonella typhi	16	14	12	8	**R. EA**	24
Escherichia coli	21	18	8	6	**R. MeOH**	28
Escherichia coli	6	6	6	6	**R. Hex**	28
Escherichia coli	6	6	6	6	**R. H_2O**	28
Escherichia coli	6	6	6	6	**R. EA**	28
Bacillus subtilis	21	16	12	8	**R. MeOH**	32
Bacillus subtilis	6	6	6	6	**R. Hex**	32
Bacillus subtilis	6	6	6	6	**R. H_2O**	32
Bacillus subtilis	13	12	10	6	**R. EA**	32
						Amphotericin B (10 µg)

Aspergillus flavus	6	6	6	6	**R. MeOH**	20
Aspergillus flavus	6	6	6	6	**R. Hex**	20
Aspergillus flavus	6	6	6	6	**R. H_2O**	20
Aspergillus flavus	6	6	6	6	**R. EA**	20
Trichophyton rubrum	6	6	6	6	**R. MeOH**	22
Trichophyton rubrum	6	6	6	6	**R. Hex**	22
Trichophyton rubrum	6	6	6	6	**R. H_2O**	22
Trichophyton rubrum	6	6	6	6	**R. EA**	22
						Fluconazole (20 mg)
Candida albicans	10	8	6	6	**R. MeOH**	18
Candida albicans	6	6	6	6	**R. Hex**	18
Candida albicans	6	6	6	6	**R. H_2O**	18
Candida albicans	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.H_2O 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)

	CONCENTRATION OF EXTRACT (mg/ml)									
ORGANISM	**400**	**200**	**100**	**50**	**25**	**12.5**	**6.25**		**EXTRACT**	**MIC (mg/ml)**
Staphylococcus aureus	-	-	-	-	-	+	+		**R. MeOH**	25
	-	-	- µ	+	µ	+	+		**R. H_2O**	100
Staphylococcus aureus	-	- µ	+	+	+	+	+		**R. EA**	200
Staphylococcus aureus					+					
Klebsiella pneumonia	-	- µ	+	+	+	+	+		**R. MeOH**	200
	-	-	- µ	+	+	+	+		**R. H_2O**	100
Klebsiella pneumonia										
Salmonella typhi	-	-	-	-	- µ	+	+		**R. MeOH**	25
Salmonella typhi	-	-	- µ	+	+	+	+		**R. H_2O**	100
Salmonella typhi	-	- µ	+	+	+	+	+		**R. EA**	
Escherichia coli	-	-	- µ	+	+	+	+		**R. MeOH**	100
Bacillus subtilis	-	- µ	+	+	+	+	+		**R. MeOH**	200

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

Table 4: MINIMUM BACTERICIDAL CONCENTRATION (MBC)

	CONCENTRATION OF EXTRACT (mg/ml)									
ORGANISM	**400**	**200**	**100**	**50**	**25**	**12.5**	**6.25**		**EXTRACT**	**MBC (mg/ml)**
Staphylococcus aureus	-	-	- β	+	+	+	+		**R. MeOH**	100
Staphylococcus aureus	-	- β	+	+	+	+	+		**R. H_2O**	200
Staphylococcus aureus	- β	+	+	+	+	+	+		**R. EA**	400
Klebsiella pneumonia	- β	+	+	+	+	+	+		**R. MeOH**	400
Klebsiella pneumonia	+	+	+	+	+	+	+		**R. H_2O**	0
Salmonella typhi	-	- β	+	+	- μ	+	+		**R. MeOH**	200
Salmonella typhi	- β	+	+	+	+	+	+		**R. H_2O**	400
Salmonella typhi	-	- μ	+	+	+	+	+		**R. EA**	200
Escherichia coli	-	- β	+	+	+	+	+		**R. MeOH**	200

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

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 and100 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 *Anogeisusslieocarpus*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 *Anogeisussleiocarpus*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.

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