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# Antimicrobial and Phytochemical Screening of Different Fractions of *Anogeisuss Leiocarpus* Guill and Perr Leaf Obtained From Langtang, Plateau State, Nigeria

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**Abstract**

*Anogeissus leiocarpus* plant is widely used in Africa and among the Tarok people in the Northern Senatorial Zone of Plateau for antimicrobial activities against many pathogenic microorganisms for Treatment many diseases. This study was carried out in vitro to compare the antibacterial and antifungal activities of non-polar and polar leaf extracts from Langtang against *Staphylococcus aureus* (clinical isolate), *Klebsiella pneumonia* (clinical isolate), *Salmonella typhi* (clinical isolate), *Escherichia coli* (clinical isolate), *Aspergillus flavus* (clinical isolates), *Trichophyton rubrum* (clinical isolates), *Aspergillus braziliensis* (clinical isolates), and *Candida albicans* (clinical isolate). The extracts of the ethyl acetate showed stronger antibacterial activity against *Staphylococcus aureus* (21 mm), *Klebsiella pneumonia* (19 mm) and *Salmonella typhi* (23 mm). Thus, zone of inhibition range is 19 -23 mm. However, methanol fraction showed slightly stronger activity against *Escherichia coli* (22 mm). The methanol fraction showed stronger inhibition against *Aspergillus flavus* (12 mm), *Trichophyton rubrum* (18 mm), *Aspergillus braziliensis* (15 mm) which range is 12 -18 mm. But for *Candida albicans*, the ethyl acetate fraction inhibition zone is 17 mm while methanol fraction is 13 mm. Gentamicin, Fluconazole and Amphotericin B were used as positive control and showed strong inhibition against the organisms with Amphotericin B with stronger MIC and MBC/MFC at 0.01 mg/ml. The result of phytochemical screening showed that the plant's leaf contained important secondary metabolites such as cardiac glycosides, tannins, saponins, steroids, carbohydrates, flavonoids and terpenes. These phytochemicals may be responsible for the antibacterial activity of this plant leaf and could be utilized in the search for new antibiotics.

**Keywords**: *Anogeissus leiocarpus*, clinical isolates, antibacterial activity, secondary metabolites, Polar Fractions, Tarok.

## Introduction

In Africa, our forefathers were known for using plants for the treatment of various diseases [1]. One such plant is *Anogeisuss leiocarpus* popularly known as marke in Hausa. Both the Sudanese and Taroke people of northern senatorial of Plateau state use this plant for traditional medicine and is well known for its antimicrobial activities against many pathogenic micro-organisms 'that caused different diseases[1,2]. These diseases include;

toothache, diarrhea, respiratory diseases, jaundice, hepatitis, haemorrhoids, headache and malaria [3] skin diseases and infections, wounds infections, sore feet, boils, syphilitic and diabetic ulcers [4].

*Anogeisuss leiocarpus* belongs 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 [5]. Other compounds include ellagitannins and stilbenes[6]. The genius of Anogeisuss also contain the following metabolites with antimicrobial activities; tnnins, polyphenol, flavonoids, steroids, stilbenes and liginan[7].

The purpose of this research is to identify the active compounds in the polar and non-polar fractions of the plant leaf extracts that are responsible for Antimicrobial property against the selected eight test organisms (Staphylococcus aureus, Klebsiella pneumonia, Salmonella typhi, Escherichia coli, Aspergillus flavus, Trichophyton rubrum, and Candida albicans)[2] which caused are responsible for many disease such as s toothache, diarrhea, respiratory diseases and skin diseases.

## Materials and Methods

The following materials were used for the research; Hexane, ethyl acetate, methanol of ASTM grade of 99.85 % and water. (ii) Nutrient agar (Oxoid, UK, CM0017B) (iii) Gentamycin,
Amphotericin and Fluconazole.

### Sample collection and preparation

The leaves of Anogeisuss leiocarpus was collected from Lantang L.G.A in southern senatorial zone of Plateau State. And was taken to the Federal College of Forestry Jos, Jos Plateau State for identification and authentication by Mr. Christopher Abok. A voucher specimen (FHJ839) was deposited in the Herbarium unit of the college. It was then washed under running water and air dried for 72 hours, ground and sieved using 30 mesh or < 1.0 mm size screen. Successive extraction of 500 grams of the leaf samples was carried out starting with 1000 $cm^3$ non-polar solvent (Hexane) and then polar solvents (ethyl acetate, methanol and water) for 6 h each. The samples were then packaged for further analysis.

### Sample analysis:

(a) **Microorganisms**

The antimicrobial activity of the plant extract was evaluated using four bacterial isolates (*Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhi*-clinical isolates and *Escherichia coli*) and four fungal isolates (*Candida albicans*, *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**

The pure culture of each organism was selected. A 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 ºC 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 inoculum or 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**

The Agar well (ditch) diffusion method was used. The method was carried out as described by [8,9]. Mueller-Hinton agar (MHA) and Sabouraud dextrose agar plates were prepared according to the manufacturer's instruction. They were incubated for sterility check at 37 ºC and 25 ºC 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 the remaining well, Gentamycin (20 mg/ml) and Amphotericin B (20 mg/ml) were dispensed as positive controls. 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 ºC for 24 h for bacteria and 25 ºC for 4 days for fungi. The diameter of zones of inhibition was measured using a meter rule and considered an indication for antimicrobial activity.

**(**d) **Determination of minimum inhibitory concentration (MIC)**

Modified broth dilution methods as described by [8,9] 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 ºC and 25 ºC 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 organisms.

(e) **Determination of minimum bactericidal/fngicidal 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 ºC and 25 ºC for 24 h. and 3 days for bacteria and fungi respectively. The lowest concentration that yielded no single bacterial or fungal colony on the medium was taken as MBC and MFC.

**Results**:

**Table 1: Photochemical leaf analysis for different fractions** [10]

| Constituents | Hexane | Ethyl acetate | Methanol | Water |
|---|---|---|---|---|
| Alkaloids | - | - | - | - |
| Saponins | - | - | ++ | - |
| Taninns | - | ++ | +++ | +++ |
| Flavonoids | - | +++ | +++ | +++ |
| Cabohydrates | - | + | ++ | + |
| Steriods | +++ | - | + | - |
| Terpenes | +++ | +++ | + | - |
| Anthraquinones | + | - | - | - |
| Cardic glycosides | + | + | + | - |

**Key:** + present

++ Average

+++ Very present

**Table 2: Percentage Yield of Extract**

| Crude extract(WCE) | Hexane extract (600 g) | Ethyl acetate extract (600g) | Methanol extract (600 g) | Water extract (600 g) |
|---|---|---|---|---|
| Weight of extract(WE) | 9.2 g | 21.1 g | 105.8 g | 53.7 g |
| % yield of extract $\left(\frac{WE}{WCE}\times 100\right)$ | $\frac{9.2}{600}\times 100$ = 1.53 | $\frac{21.1}{600}\times 100$ = 3.52 | $\frac{105.8}{600}\times 100$ = 17.63 | $\frac{55.7}{600}\times 100$ = 9.28 |

**Table 3: Antimicrobial Activity**

| Organisms | Concentration of Extact (mg/ml)/Average Diameter 0f Zones of Inhibition (mm) | | | | Extract | Positive control |
|---|---|---|---|---|---|---|
| | 800 | 400 | 200 | 100 | | Gentamycin (20 mg/ml) |
| SA | 21 | 19 | 15 | 0 | Ethyl acetate | 28 |
| SA | 19 | 12 | 08 | | Methanol | 28 |
| SA | 0 | 0 | 0 | 0 | Hexane | 28 |
| SA | 0 | 0 | 0 | 0 | Aqueous | 28 |
| KP | 19 | 16 | 10 | 0 | Ethyl acetate | 32 |
| KP | 10 | 08 | 0 | 0 | Methanol | 32 |
| KP | 0 | 0 | 0 | 0 | Hexane | 31 |
| KP | 0 | 0 | 0 | 0 | Aqueous | 32 |
| ST | 23 | 16 | 09 | 0 | Ethyl acetate | 29 |
| ST | 12 | 10 | 0 | 0 | Methanol | 30 |
| ST | 0 | 0 | 0 | 0 | Hexane | 29 |
| ST | 0 | 0 | 0 | 0 | Aqueous | 30 |
| EC | 18 | 10 | 09 | 0 | Ethyl acetate | 32 |
| EC | 22 | 17 | 12 | 0 | Methanol | 32 |
| EC | 0 | 0 | 0 | 0 | Hexane | 32 |
| EC | 0 | 0 | 0 | 0 | Aqueous | 32 |

| | | | | | | **Amphotericin B (10 µg)** |
|---|---|---|---|---|---|---|
| AF | 10 | 0 | 0 | 0 | Ethyl acetate | 18 |
| AF | 12 | 10 | 0 | 0 | Methanol | 18 |
| AF | 0 | 0 | 0 | 0 | Hexane | 20 |
| AF | 0 | 0 | 0 | 0 | Aqueous | 20 |
| | | | | | | |
| TR | 14 | 09 | 0 | 0 | Ethyl acetate | 22 |
| TR | 18 | 19 | 0 | 0 | Methanol | 22 |
| TR | 0 | 0 | 0 | 0 | Hexane | 22 |
| TR | 0 | 0 | 0 | 0 | Aqueous | 22 |
| | | | | | | |
| AB | 12 | 10 | 0 | 0 | Ethyl acetate | 20 |
| AB | 15 | 12 | 0 | 0 | Methanol | 20 |
| AB | 0 | 0 | 0 | 0 | Hexane | 21 |
| AB | 0 | 0 | 0 | 0 | Aqueous | 21 |
| | | | | | | **Fluconazole (20 mg)** |
| CA | 17 | 12 | 09 | 0 | Ethyl acetate | 18 |
| CA | 13 | 10 | 0 | 0 | Methanol | 18 |
| CA | 0 | 0 | 0 | 0 | Hexane | 18 |
| CA | 0 | 0 | 0 | 0 | Aqueous | 18 |

**Key**: Bacteria: SA *Staphylococcus aureus*, KP *Klebsiella pneumonia*, ST *Salmonella typhi*, EC *Escherichia coli*.

Fungi: AF *Aspergillus flavus*, TR *Trichophyton rubrum*, AB *Aspergillus braziliensis*, CA *Candida albicans*.

**Table 4: Minimum Inhibition Concentration (MIC)**

| | Concentration of Extract (mg/ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Organism | 800 | 400 | 200 | 100 | 50 | 25 | 12.5 | EXTRACT | MIC (mg/ml) |
| SA | - | - | - µ | + | + | + | + | Ethyl acetate | 200 |
| SA | - | - µ | + | + | + | + | + | Methanol | 400 |
| KP | - | - µ | + | + | + | + | + | Ethyl acetate | 400 |
| KP | - µ | + | + | + | + | + | + | Methanol | 800 |
| ST | - | - | - µ | + | + | + | + | Ethyl acetate | 200 |
| ST | - µ | + | + | + | + | + | + | Methanol | 800 |
| EC | - | - µ | + | + | + | + | + | Ethyl acetate | 400 |
| EC | - | - | - µ | + | + | + | + | Methanol | 200 |
| AF | + | + | + | + | + | + | + | Ethyl acetate | 0 |
| AF | + | + | + | + | + | + | + | Methanol | 0 |
| TR | + | + | + | + | + | + | + | Ethyl acetate | 0 |
| TR | - | - µ | + | + | + | + | + | Methanol | 400 |
| AB | - µ | + | + | + | + | + | + | Ethyl acetate | 800 |
| AB | - µ | + | + | + | + | + | + | Methanol | 800 |
| CA | - µ | + | + | + | + | + | + | Ethyl acetate | 800 |
| CA | - µ | + | + | + | + | + | + | Methanol | 800 |

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

0 = no visible growth of organisms.

**Table 5: Minimum Bactericidal Concentration (MBC)**

| | Concentration of Extract (mg/ml) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Organism | 800 | 400 | 200 | 100 | 50 | 25 | 12.5 | Extract | MBC (mg/ml) |
| SA | - | - β | + | + | + | + | + | Ethyl acetate | 400 |
| SA | - β | + | + | + | + | + | + | Methanol | 800 |
| KP | - β | + | + | + | + | + | + | Ethyl acetate | 400 |
| KP | + | + | + | + | + | + | + | Methanol | 0 |
| ST | - | - β | + | + | + | + | + | Ethyl acetate | 400 |
| ST | + | + | + | + | + | + | + | Methanol | 0 |
| EC | - β | + | + | + | + | + | + | Ethyl acetate | 800 |
| EC | - | - | - β | + | + | + | + | Methanol | 200 |
| AF | + | + | + | + | + | + | + | Ethyl acetate | 0 |
| AF | + | + | + | + | + | + | + | Methanol | 0 |
| TR | + | + | + | + | + | + | + | Ethyl acetate | 0 |
| TR | - β | + | + | + | + | + | + | Methanol | 800 |
| AB | + | + | + | + | + | + | + | Ethyl acetate | 0 |
| AB | + | + | + | + | + | + | + | Methanol | 0 |
| CA | + | + | + | + | + | + | + | Ethyl acetate | 0 |
| CA | + | + | + | + | + | + | + | Methanol | 0 |

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

**Table 6: Minimum Inhibition Concentration (MIC)**

| | | | | Gentamicin (mg/ml) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Organism | 20 | 10 | 5 | 2.5 | 1.25 | 0.625 | 0.3125 | 0.1562 | MIC (mg/ml) |
| SA | - | - | - | - | - | - | - | - µ | < 0.1562 |
| KP | - | - | - | - | - | - µ | + | + | 0.625 |
| ST | - | - | - | - | - | - | - µ | + | 0.3125 |
| EC | - | - | - | - | - | - | - | - µ | < 0.1562 |
| | | | | Amphotericin B (µg/ml) | | | | | |
| Organism | 10 | 5 | 2.5 | 1.25 | 0.625 | 0.3125 | 0.1562 | | MIC |
| AF | - | + | + | + | + | + | + | | 10 |
| TR | - | + | + | + | + | + | + | | 10 |
| AB | - | + | + | + | + | + | + | | 10 |
| | | | | Fluconazole (mg/ml) | | | | | |
| | 20 | 10 | 5 | 2.5 | 1.25 | 0.625 | 0.3125 | 0.1562 | MIC |
| CA | - | - | + | + | + | + | + | + | 10 |

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

**Table 7: Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)**

| | | | | Gentamicin (mg/ml) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Organism | 20 | 10 | 5 | 2.5 | 1.25 | 0.625 | 0.3125 | 0.1562 | MBC (mg/ml) |
| SA | - | - | - | - | - | - | - | - β | < 0.1562 |
| KP | - | - | - | - | - | - β | + | + | 0.625 |
| ST | - | - | - | - | - | - | - β | + | 0.3125 |
| EC | - | - | - | - | - | - | - β | + | 0.3125 |
| | | | | Amphotericin B (µg/ml) | | | | | |
| Organism | 10 | 5 | 2.5 | 1.25 | 0.625 | 0.3125 | 0.1562 | | MFC |
| AF | - β | + | + | + | + | + | + | | 10 |
| TR | - β | + | + | + | + | + | + | | 10 |
| AB | - β | + | + | + | + | + | + | | 10 |
| | | | | Fluconazole (mg/ml) | | | | | |
| | 20 | 10 | 5 | 2.5 | 1.25 | 0.625 | 0.3125 | 0.1562 | MFC |
| CA | - β | + | + | + | + | + | + | + | 20 |

Key: - = No turbidity, + =Turbidity, β = MBC/MFC

**Discussion**

This study was carried out in vitro to compare the antibacterial and antifungal activities of non-polar (hexane,) and polar (ethyl acetate,methanol, water) leaf extracts of Anogeisuss leiocarpus from Langtang against Staphylococcus aureus (clinical isolate), Klebsiella pneumonia (clinical isolate), Salmonella typhi, Escherichia coli (clinical isolate), Aspergillus flavus, Trichophyton rubrum, Candida albicans (clinical isolate). Table 1 shows the presence of some very important secondary metabolites such as terpenoids, alkaloids, flavonoids and tannins [12,13]. Investigation revealed that terpenoids have strong microbial inhibition among other secondary metabolites [12]. Terpenoids are a major source of bioactive natural products. Especially because of their lipophilic characteristics, terpenoids have become one of the major kinds of antimicrobial agents against various microorganisms [14]. The ethyl acetate fraction has a

very high presence of terpenoids compared to other fractions. This result may be the reason for the inhibition against bacterial such as *Staphylococcus aureus*, *Klebsiella pneumonia* and *Salmonella typhi* as shown in Table 3. In addition, the presence of Saponins in the methanol extracts may be responsible for activity against fungi such as *Aspergillus flavus*, *Aspergillus braziliensis* and Trichophyton rubrum. The zone inhibition is between 12 – 18mm [15]. However, the ethyl acetate fraction showed slightly stronger activity than the methanol fraction against Candida albicans.

Table 4, shows that MIC for bacteria is between 200 -800 mg/ml (ethyl acetate fraction) and 400 – 800 mg/ml (methanol fraction). The MIC for Aspergillus flavus is zero for both fractions (ethyl acetate and methanol extract). The fungi, Trichophyton rubrum, is zero for ethyl acetate extract but 400 mg/ml for methanol extract. For *Aspergillus braziliensis*, and *Candida albicans*, MIC is 800 mg/l for both extracts. The presence of turbidity means no activity while the absence means activity against organisms. According to Ikram et al. (2015), revealed that both ethyl acetate and methanol extract showed stronger activity among four fractions (extract) used in their study. All organisms used were susceptible to both extracts. Thus, in agreement with this present study.

Although some studies have showed that organisms like *Salmonella coli*, *Klebsiella pneumonia* and *Candida albicans* were not susceptible to the crude methanol stem bark extract [15]. However, the present study has shown that the leaf extract is potent against these organisms. Table 5, presents the minimum bactericidal concentration (MBC) for the different fractions. The difference between MIC and MBC is that the former aim at inhibiting organism growth without necessarily killing the organisms.

The MBC for the ethyl acetate fraction is either 400 mg/ml or 800 mg/ml for all the bacteria except Escherichia coli and Staphylococcus aureus which is 200 and 800 mg/ml respectively. The MBC for *Klebsiella pneumonia* and *Salmonella typhi* is zero meaning that even at 800 mg/ml there was bacterial growth. The MBC for the fungi shows that the methanol extract killed 99.9 % of Trichophyton rubrum at 800 mg/ml; while the other fungi were not eliminated by both fractions. Tables 6 and 7 shows the activity of standard drugs; Gentamicin, Amphotericin and Fluconazole against the organisms used in this research. They all exhibited activities against both the bacteria and fungi used in this study. However, the Amphotericin B shows stronger inhibition (MIC) and MBC/MFC at 0.01 mg/ml (10 μg/ml). Gentamicin MIC and MBC/MFC are < 0.1562 mg/ml and Fluconazole is 10 mg/ml for both parameters.

**Conclusion**

The plant leaf extracts of *Anogeisuss leiocarpus* contain bioactive compounds that could be used as lead compounds in the search for new drugs to replace drugs used in the treatment of diseases coursed by some bacteria and fungi[2,5,,16,]. This research may justify some of the traditional usage

of this plant most especially in diseases caused by the tested organisms.

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