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*Full Length Research Paper*

# Phytochemical and antibacterial screening of *Anogeissus leiocarpus* against some microorganisms associated with infectious wounds

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**Ethanolic extracts of the leaf, stem and root bark and the combination of the three parts of *Anogeissus leiocarpus* were investigated for *in vitro* antibacterial activity against clinical isolates of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* using agar diffusion techniques. The extracts of the plant parts showed higher antibacterial activity against *S. aureus* (15.80 ± 0.85) than other tested organisms. The plant parts generally were found to contain important bioactive substances such as glycosides, phenols, tannins, saponins, alkaloids, steroids, ellagic acids and anthraquinones. These agents may be responsible for the antibacterial activity of this plant.**

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

## INTRODUCTION

Plant-derived compounds are a major area of interest to source for safer and more effective antibacterial agents (Baladrin et al., 1985). *Anogeissus leiocarpus* (DC) Guill and Perr family Combretaceae (Common name: Axle-wood tree) has many applications in Nigeria. *A. leiocarpus* is used medically for the treatment of ascaricide, gonorrhoea, general body pain, blood clots, asthma, coughing and tuberculosis (Mann et al., 2003). Information obtained from the Yorubas and South-Eastern people of Nigeria illustrate that the plant is also used as an antimicrobial agent against bacterial infections (Dweek, 1996). The leaves of the plant are used externally as a decoction in the eastern part of Nigeria for the treatment of skin diseases and the itch of psoriasis. The powdered bark is applied to wounds, sores, boils, cysts and diabetic ulcers with good results. The powdered bark has also been mixed with 'green clay' and applied as an unusual face mask for serious blackheads (Dweek, 1996). The infusion and decoctions are used as cough medicine, the pulped roots are applied to wounds and ulcers, the powdered bark is also rubbed to reduced tooth ache on gums, it is also used as vermifuges and the leaves decoction is used for washing and fumigation (Ibrahim et al., 1997). *A. leiocarpus* is traditionally ac-claimed to be effective in treating infectious wounds in man and animals (Dweek, 1996). This paper seeks to investigate whether the extracts of *A. leiocarpus* could inhibit microorganisms that are incriminated in the pathogenesis of infectious wounds. Thus, the extracts of the leaf, stem and root bark of A. *leiocarpus* were screened for antibacterial activity against three clinical isolates.

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## MATERIALS AND METHODS

### Plant material

The root, stem bark and leaves of *A. leiocarpus* used in this study were collected in the Federal Polytechnic Bida, Niger state, Nigeria. The plant was authenticated by Muhammad Musa at the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria where a voucher specimen was deposited in the Herbarium with the Herbarium number ABUHH 167.

### Extraction of plant materials

The leaf, root and stem bark was first air-dried at ambient temperature for 4 weeks and pulverized to powder using a clean electric blender (Phillips 190). The leaf, root and stem bark of *A. leiocarpus* were prepared by percolation in ethanol. A 50.0g sample of each of the pulverized plant parts were separately soaked in 200 ml of ethanol (0.25 g/ml) and allowed to stand for 72 h with intermittent stirring. Each preparation of the leaf, root and stem bark of *A. leiocarpus* was filtered through a Whatman No 1 filter paper and the filtrates obtained were evaporated to dryness using a rotary evaporator at 35$^{O}$C to give 7.6, 8.1, 10.3 g, respectively.

**Table 1.** Phytochemical screening of *Anogeissus leiocarpus* extracts.

| Plant part | Alkaloid | Glycoside | Steroid | Phenol | Tannins | Ellagic acid | Anthraquinones | Saponins | Flavonoid |
|---|---|---|---|---|---|---|---|---|---|
| Leaves | + | + | - | + | + | - | - | + | + |
| Root barks | + | + | + | + | + | + | + | + | + |
| Stem barks | + | + | + | - | - | + | + | + | + |

(+) = Present; (-) = absent

**Table 2.** Inhibition of S*taphylococcus aureus* by different extract concentrations of *Anogeissus leiocarpus*.

| Concentration (mg/ml) | | | Mean diameter of zone of inhibition (mm) ± S.D | | | |
|---|---|---|---|---|---|---|
| | DMSO | Control | Root | Stem | Leaves | Root/ stem + leaves |
| 10 | NZI | 2.70 ± 0.24 | 3.00 ±0.00 | 3.00 ±0.64 | NZI | NZI |
| 20 | NZI | NT | 5.00 ±1.25 | 5.80 ± 1.25 | NZI | 3.70 ± 0.62 |
| 30 | NZI | NT | 10.80 ± 0.48 | 10.80 ± 0.48 | 0.90 ± 0.62 | 5.50 ± 0.52 |
| 40 | NZI | NT | 14.30 ± 0.30 | 11.90 ± 0.20 | 5.30 ± 0.11 | 2.10 ± 0.33 |
| 50 | NZI | NT | 18.10 ± 1.08 | 13.70 ± 0.10 | 9.00 ± 0.47 | 15.80 ± 0.85 |

S.D: Standard deviation; NZI: No zone of inhibition; NT: Not Tested; DMSO: Dimethylsulphoxide; Values are mean ± standard deviation; Values greater than 6.0 mm indicate some activity.

The dried extracts were exposed to UV rays for 24 h and checked for sterility by streaking on nutrient agar plate. The extracts were assayed against the test organisms to determine the antimicrobial properties (Bauer et al., 1966).

#### Phytochemical screening

The extracts were subjected to phytochemical analysis using procedures of Harbone (1998) and Evans (1998).

#### Test microorganisms

Clinical isolates of S*taphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the Medical Microbiology Laboratory of the Federal Medical Centre, Bida, Niger state. The organisms were maintained on nutrient agar slants and refrigerated until required for use.

#### Antibacterial activity

The ethanolic leaf, root bark and stem bark were spot checked for antibacterial activity using the agar well diffusion technique (Bauer et al., 1966). Standardized inoculum ($5 \times 10^5$ cfu/ml) of each test bacterium was spread onto sterile nutrient agar plates so as to achieve even growth. The plates were allowed to dry and a sterile cork borer of diameter 6.0 mm was used to bore wells in the agar plates. The extracts were prepared by first reconstituting in 20% dimethylsulphoxide (DMSO). They were diluted in sterile distilled water to achieve different concentrations of 10, 20, 30, 40, and 50 mg/ml. Subsequently, a 100 µl volume of the extracts were introduced in triplicate wells in the nutrient agar plate cultures. The prepared antibiotic (Tetracycline at 10 mg/ml; Joabez Corporation) was used as a positive control while the negative control was 20% nutrient agar. The plates were then incubated at $37^{o}$C for 24 h. The were allowed to stand for 1 h to allow diffusion of the extracts into dimethylsulphoxide (DMSO) in sterile distilled water. The plates the antimicrobial activity of the extracts was determined in terms of zones of inhibition.

## RESULTS

The phytochemical screening of the extracts of A*. leiocarpus* indicated presence of alkaloids, glycosides, phenols, steroids, tannins, ellagic acids, anthraquinones, saponins and flavonoids (Table 1). Table 2 shows the diameter of zones of inhibition against *S*. *aureus.* Combinations of the root, stem bark and leaves of A*. leiocarpus* showed the highest inhibition against the test organisms. Table 3 shows the diameter of zones of inhibition against *E. coli* when the extract of *A. leiocarpus* was assayed against the test organism. Low concentrations (10 mg/ml) of the root extract exhibited antibacterial activity against the organism in zone diameter studies. Table 4 shows the diameter of zone of inhibition against *P. aeruginosa*. A combination of root bark, stem bark and leaves of A*. leiocarpus* showed the highest diameter of inhibition.

Tables 2 and 3 also show the diameter of zones of inhibition, when Tetracycline was assayed against the test organisms, with their susceptibility decreasing from S*. aureus* to *E. coli*. The antibiotic showed no antibacterial activity against *P. aeruginosa* (Table 4).

## DISCUSSION

The results showed that extracts of the plant parts had exhibited antibacterial activity against S*. aureus*, *E. coli* and *P. aeruginosa*. It was revealed that the antibacterial activity of the extracts was enhanced by an increase in

**Table 3.** Inhibition of *Escherichia coli* by different extract concentrations of *Anogeissus leiocarpus.*

| Concentration (mg/ml) | Mean diameter of zone of inhibition (mm) ± S.D | | | | | |
|---|---|---|---|---|---|---|
| | DMSO | Control | Root | Stem | Leaves | Root/ stem + leaves |
| 10 | NZI | 1.50± 0.63 | 1.30 ±0.00 | NZI | NZI | NZI |
| 20 | NZI | NT | 2.10±1.63 | NZI | NZI | 2.50±1.08 |
| 30 | NZI | NT | 5.50±0.26 | NZI | NZI | 3.90±0.00 |
| 40 | NZI | NT | 8.80±0.20 | 5.30±0.73 | 2.80±0.85 | 7.30±0.63 |
| 50 | NZI | NT | 11.80±0.44 | 9.10±0.94 | 4.80±0.92 | 12.60±1.82 |

S.D: Standard deviation; NZI: No zone of inhibition; NT: Not Tested; DMSO: Dimethylsulphoxide; Values are mean ± standard deviation; Values greater than 6.0 mm indicate some activity.

**Table 4.** Inhibition of *Pseudomonas aeruginosa* by different extract concentrations of *Anogeissus leiocarpus*

| Concentration (mg/ml) | Mean diameter of zone of inhibition (mm) ± S.D | | | | | |
|---|---|---|---|---|---|---|
| | DMSO | Control | Root | Stem | Leaves | Root/ stem + leaves |
| 10 | NZI | NZI | NZI | NZI | NZI | NZI |
| 20 | NZI | NT | NZI | NZI | NZI | NZI |
| 30 | NZI | NT | 2.70±0.24 | 2.90±0.82 | NZI | 5.80±0.82 |
| 40 | NZI | NT | 6.40±0.34 | 5.00±0.44 | NZI | 9.80±0.00 |
| 50 | NZI | NT | 9.00±1.40 | 7.60±0.78 | NZI | 12.90±1.63 |

S.D: Standard deviation; NZI: No zone of inhibition; NT: Not Tested; DMSO: Dimethylsulphoxide; Values are mean ± standard deviation; Values greater than 6.0 mm indicate some activity.

the concentration of the extracts (Tables 2, 3 and 4). This finding agrees with the report of Banso et al. (1999), i.e. that higher concentration of antimicrobial substances showed appreciable growth inhibition. The zones of inhibition produced by the test organisms indicated their susceptibility to the plant extracts; it was observed that the zones of inhibition varied from one organism to another and from one plant part extract to another. According to Prescott (2002) the effect of an agent varies with target species. Hugo and Russell (1998) also reported that the position of the zone edge (diameter of zone of inhibition) is determined by the initial population density of the organism, their growth rate and the rate of diffusion of the anti microbial agent. This explains the differences in the zones of inhibition observed. The extract from the root and stem bark and the combination of the various parts of the plant were found to exhibit antibacterial activity against the test organisms. This justifies their traditional usage as medicinal plant amongst the peoples of Niger state. This may be due to the presence of the active principles observed. Secon-dary plant metabolites constitute an important source of microbiocides, pesticides and many pharmaceutical drugs (Ibrahim et al., 1997; Kolapo et al., 2007). Ethanol extracts of the plant parts showed antibacterial activity against disease-causing organisms. These observations suggest that constituents of the plant parts could be useful in chemotherapy. The isolation of the active anti - bacterial agent(s) and toxicological studies of the parts of the plant are recommended.

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