

Antibacterial Activity of Terpenoidal Fractions From *Anogeissus* Against Community Acquired Infections

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Abstract: Terpenoidal fractions were isolated from both *Anogeissus leiocarpus* (DC) Guill and Perr (Stem) and assayed against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The terpenoidal fractions exhibited antimicrobial activities against all the test microorganisms. All test organisms were susceptible to the terpenoidal fractions. The minimum inhibitory concentration ranged between 0.213 and 5.0 µg/ml. The terpenoidal fractions from *A. leiocarpus* could be a potential source of chemotherapeutic agents. The antimicrobial activities of these terpenoidal fractions provide justification for the chemotherapeutic utilization of these plants.

Keywords: *Anogeissus leiocarpus*, Terpenoidal fractions, antimicrobial activities.

1. Introduction

Infective diseases account for approximately one-half of all death in tropics (Iwu et al., 1999). In the area of anti-infectives about 70% are naturally derived (Cragg and Newman, 2005). The screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic chemotypes. Nigeria's diverse flora offers a wide spectrum of medicinal plants. Many Combretaceae species are widely distributed in Nigeria and are used in traditional medicine for treating of respiratory diseases (asthma, catarrh, chronic bronchitis, cough, hay-fever, hemoptysis, pneumonia, pulmonary disorders and tuberculosis) (Mann et al., 2007) and other human diseases.

Some members of the Combretaceae have high concentrations of flavonoids, terpenoids, tannins or polyphenolic compounds. These compounds are known to have *in vitro* antimicrobial activity (Adigun et al., 2000; Sofowora, 1969; Mann et al., 2008). *Anogeissus* Adigun et al., 2001; Almagboul et al., 1988; Malcolm and leiocarpus (DC) Guill and Perr has been shown to be active as antimicrobial agent against gram-positive and gram-negative bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Adeleye et al., 2003; Ibrahim et al., 2005; Machado and Ado, 1999; Ndukwe et al., 2005; Taiwo et al., 1999); anti-mycobacterial activity (Malcolm and Sofowora, 1969; Johnbull and Abdu, 2006; Uba et al., 2003); trypanocidal activity (Atawodi et al., 2003) and demonstrated activity against *Candida albicans* (Chaabi, et al., 2006; Sanogo et al., 1997; Sanogo, 2005).

Previous studies showed that the bark extract of *Terminalia avicennioides* Guill and Perr exhibited vibriocidal and typhoidal activities (Akinsinde and Olukoya, 1995; Akinyemi et al., 2000) as well as anti-methicillin resistant *S. aureus* activity (Akinyemi et al., 2005), while the aqueous extracts of the roots exhibited anti-diarrheal activity (Abdullahi et al., 2001).

It is therefore of interest to investigate antimicrobial activities of the terpenoidal fractions of the two plants commonly used for the treatment of respiratory and other related ailments among indigenous people.

2. Materials and Methods

2.1 Plant materials

Leiocarpus (DC) Guill and Perr (Stem) used were obtained as described by traditional medical practitioners from a forest. Voucher specimens were deposited in the Herbarium at the Department of Biological Sciences

2.2 Extraction and isolation

Dried and ground plant materials (5 kg) were successively macerated with *n*-hexane, ethyl acetate, acetone and methanol. Each extract was concentrated *in vacuo* to dryness yielding: *A. leiocarpus* A1 (0.32), A1 (6.22), A1 (6.88), and A1 (4.8), respectively. Antimicrobial screening of the extracts led to further investigation of the *n*-hexane and ethyl acetate extracts. EtOAc extract of A1 (brown solid, 30 g) was subjected to Flash Column Chromatography (FCC) (150 g, Si gel 60HF254+366) and eluted successively with gradient mixtures of *n*-hexane, EtOAc and MeOH. Fractions were combined based on the Thin Layer Chromatography (TLC) behaviour to yield AIF1 (F1-8), AIF9 (F9-11), AIF12 (F12-19), AIF20 (F20-34), AIF35 (F35-40), AIF41 (F41-45), AIF46 (F46-52) and AIF53 (F53-62). AIF12 which was eluted with *n*-hexane-EtOAc (3:1 - 3:2) was subjected to CC (10 g, sephadex LH20) by elution with MeOH. Fractions labelled AIF2 (F2-5), AIF6 (F6-7), AIF8 (F8-12), AIF15 (F15-17), AIF18 (F18), AIF33 (F33), AIF36 (F36-44) and AIF45 (F45) were obtained and purified by repeated Preparative Thin Layer Chromatography (PTLC) (0.25 mm) using EtOAc/ MeOH/AcOH (94.5:5:0.5). The resulting fractions 8-12 labelled as AIF8 and 36-44 labelled as Alee gave creamy powder and white crystal respectively. EtOAc extract of Ta (brown solid, 30 g) was subjected to FCC (150 g, si gel 60HF254+366) and eluted successively with

gradient mixtures of *n*-hexane, EtOAc and MeOH. The fraction labelled F5 which eluted with *n*-hexane- EtOAc (4:1) was subjected to Column Chromatography (CC) (10 g, sephadex LH20) by elution with MeOH. The fractions labelled TaF5A (F10-95) and TaF5B (F100-116) were obtained as whitish yellow and white powder respectively. Similarly *n*-hexane extract of Ta (oily, 1.3 g) was ran on CC (150 g, si gel 60HF254+366) and eluted with PE-EtOAc (3:2). Furthermore, *n*hexane extract of Ta (oily, 1.08 g) was also subjected to CC (30 g, superfine sephadex) by elution with MeOH. The resulting fractions were combined based on the TLC behaviour. Fractions TaF5A, TaF1 (F1-9), TaF10 (F10-16), TaF17 (F17-19), TaF20 (F20-30), TaF31 (F31-46), TaF47 (F47-77), TaF79 (F79-96), TaF97 (F 97-110) and TaF111

(F111-126) were purified by repeated PTLC (0.25 mm) using EtOAc/ MeOH/AcOH (94.5:5:0.5). The resulting

2.5 Inocula preparation

A 1:10 dilution of 24 h culture of the test microorganism was made. Broth was used to adjust the diluted culture until the turbidity compared with McFarland standard number 0.5.

2.6 Determination of MIC of pure compounds

The MIC values were determined according a modified method (Bauer et al., 1966). The microplate (80 wells) was marked into 3 rows. Each row of 10 wells corresponds to each test microorganism used. 50 µl of broth was dispensed into microplate wells 2 - 7. 100 µl of the stock solution of extract was dispensed into well 1. From well 1, a 1:2 serial dilution was carried out by transferring 50 µl of stock solution to well 2 and mixed by aspiration and dispensing several times. From well 2, transfer 50 µl to well 3, step 5 above was repeated for wells 3 through to well 7. 50 µl of the mixture was discarded from well 7. 50 µl of test microorganism was each dispensed into wells 1 to 7. Controls were set such that 50 µl of stock, test microorganism and broth were dispensed into wells 8, 9 and 10 respectively. The volumes in wells 8, 9 and 10 were made up to 100 by 50 µl of broth. Well 8 is drug sterility, well 9 is organism viability and well 10 is media sterility. The above procedure was duplicated. The above procedure was carried out for each of the extracts. All inoculated microplates were properly labelled and incubated at 37°C for 24 h. At the end of 24 h incubation growth (turbidity in broth) was observed in wells 1 - 7 and compared with the controls in wells 8, 9 and 10.

3. Results and Discussion

The phytochemical screening of the fractions of both plants indicated presence terpenes (Table 1).

3.1 Determination of antimicrobial activity

The antimicrobial activities of terpenoidal fractions from *A. leiocarpus* (Stem) were determined using some standard microorganisms (Table 2). The MIC of terpenoidal fractions were found to range from 0.213 to 4.05213 µg/ml against *P.*

fractions 10 - 16 and 20 -30 gave creamy powder and whitish crystal respectively. Finally, further purification by repeated PTLC gave the following fractions: Ta1, Ta3, Ta4, Ta5, Ta6, Ta12, Alee, ALFA and ALF3 used in this study.

2.3 Test microorganisms

S. aureus, *Escherichia coli* and *P. aeruginosa* were used for testing. Bacteria were cultured and checked for purity at Department of Microbiology and Biotechnology, and maintained in a slant of Blood agar base.

2.4 Preparation of stock solutions

For example, 1.7 mg of Ta1 was dissolved in 250 ml of Dimethylsulphoxide (DMSO) to give a concentration of 6.8 µg/ml

aeruginosa, those of *S. aureus* are between 0.425 and 2.5 µg/ml; while *E. coli* has the MIC of range 0.425 to 5.0 µg/ml. Fractions Ta1 and Ta6 exhibit the highest activity against all the test organisms. The lowest MIC values of fractions against all the test organisms (Table 2) suggest that these fractions were most effective (Table 2). In particular, Ta1 has the highest antimicrobial activity for all three organisms tested. Fractions Ta4, Ta5 and ALFA did exhibit any activity (Table 2). The results of the present investigation clearly demon

Table 1: Phytochemical screening results of the pure compounds

Pure compound	Alkaloids	Anthraquinone	Carbohydrate	Flavonoid	Steroids	Tannin	Terpenoids
Ta1	-	-	-	-	-	-	+
Ta3	-	-	-	-	-	-	+
Ta4	-	-	-	-	+	-	+
Ta5	-	-	-	-	+	-	+
Ta6	-	-	-	-	-	-	+
Ta12	-	-	-	-	-	-	+
Alee	-	-	-	-	+	-	+
ALFA	-	-	-	-	+	-	+
ALF3	-	-	-	-	-	-	+

(+)-Present, (-) – Absent

Table 2: MIC of the various pure compounds against the test microorganisms.

Pure compound µg/ml	Staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa
Ta1	0.405	0.405	0.231
Ta3	-	1.5	1.25
Ta4	NA	NA	NA
Ta5	NA	NA	NA
Ta6	-	0.425	0.625
Ta12	2.05	2.05	4.05
Alee	2.5	4.25	2.5
TaFL	NA	NA	NA
ALF3	-	2.5	2.5

Those terpenoidal fractions from these plants possess significant *in vitro* antimicrobial activities against some of the bacteria implicated in the pathogenesis of human infections. Some infections such as: respiratory tract inflammations caused by *Pseudomonas* spp. are often

difficult to -, but the growth of these organisms was greatly inhibited by fractions from both plants (Table 2). While *E. coli* incriminated as the causative agent of gastro-intestinal and also causes infections in the lungs especially in immune deficient patients was susceptible to fractions Ta1, Ta3, Ta6, Ta12, Alee and ALF3.

It is a common practice among the traditional healers to prepare an infusion of *A. leiocarpus* separately to relieve acute respiratory tract infections, fever, cough and stomach pains. The susceptibility of these microbes to these fractions of these plants may be a pointer to their potentials as drugs that can be used against these organisms.

4. Conclusion

The present findings further confirm the efficiency of these fractions against respiratory and other related infections particularly those caused by the test organisms susceptible to these fractions. This suggests that terpenoidal fractions of these plants could be a source of new antimicrobial agents. It also forms the basis for further investigation and structural determination of the most promising fractions for *in vivo* evaluation of toxicity of these constituents in animal and human studies.

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