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Full Length Research Paper

Antibacterial activity of terpenoidal fractions from *Anogeissus leiocarpus* and *Terminalia avicennioides* against community acquired infections

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Terpenoidal fractions were isolated from both *Anogeissus leiocarpus* (DC) Guill and Perr (Stem) and *Terminalia avicennioides* Guill and Perr (Root) 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* and *T. avicennioides* could be a potential source of chemotherapeutic agents. The antimicrobial activities of these terpenoidal fractions provide justification for the chemotherapeutic utilization of these plants.

Key words: *Anogeissus leiocarpus*, *Terminalia avicennioides*, Terpenoidal fractions, antimicrobial activities.

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 have *in vitro* antimicrobial activity (Adigun et al., to 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; Machido and Ado, 1999; Ndukwe et al., 2005; Taiwo et al., 1999); antimycobacterial 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 vibrocidal 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 antidiarrhoeal 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 of Niger state, Nigeria.

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MATERIALS AND METHODS

Plant materials

A. leiocarpus (DC) Guill and Perr (Stem) and *T. avicennioides* Guill and Perr (Root) used were obtained as described by traditional medical practitioners from a forest near Baddegi, Niger State, Nigeria. Voucher specimens were deposited in the Herbarium at the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria, and National Institute for Pharmaceutical Research and Development (NIPRD) with the Herbarium numbers ABUHH 167 and NIPRDH 5735 respectively.

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* Al (0.32), *T. avicennioides* Ta (0.30); Al (6.22), Ta (8.56); Al (6.88), Ta (7.56) and Al (4.8), Ta (15.36) % (w/w) respectively. Antimicrobial screening of the extracts led to further investigation of the *n*- hexane and ethyl acetate extracts. EtOAc extract of Al (brown solid, 30 g) was subjected to Flash Column Chromatography (FCC)
(150 g, Si gel 60HF$_{254+366}$) and eluted successively with gradient mixtures of n-hexane, EtOAc and MeOH. Fractions were combined based on the Tin Layer Chromatography (TLC) behaviour to yield AlF1 (F$_{1-8}$), AlF9 (F$_{9-11}$), AlF12 (F$_{12-19}$), AlF20 (F$_{20-34}$), AlF35 (F$_{35-40}$), AlF41 (F$_{41-45}$), AlF46 (F$_{46-52}$) and AlF53 (F$_{53-62}$). AlF12 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 AlF2 (F$_{2-5}$), AlF6 (F$_{6-7}$), AlF8 (F$_{8-12}$), AlF15 (F$_{15-17}$), AlF18 (F$_{18}$), AlF33 (F$_{33}$), AlF36 (F$_{36-44}$) and AlF45 (F$_{45}$) 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 AlF8 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 60HF$_{254+366}$) and eluted successively with gradient mixtures of n-hexane, EtOAc and MeOH. The fraction labelled F$_5$ 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 (F$_{10-95}$) and TaF5B (F$_{100-116}$) 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 60HF$_{254+366}$) 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 (F$_{1-9}$), TaF10 (F$_{10-16}$), TaF17 (F$_{17-19}$), TaF20 (F$_{20-30}$), TaF31 (F$_{31-46}$), TaF47 (F$_{47-77}$), TaF79 (F$_{79-96}$), TaF97 (F $_{97-110}$) and TaF111 (F$_{111-126}$) were purified by repeated PTLC (0.25 mm) using EtOAc/ MeOH/AcOH (94.5:5:0.5). The resulting 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.

Phytochemical analysis of fractions

The plant fractions were phytochemically screened using standard techniques (Brain and Turner, 1975).

Test microorganisms

S. aureus, Escherichia coli and *P. aeruginosa* were obtained from the Clinic of National Institute for Pharmaceutical Research and Development, Abuja, Nigeria. Bacteria were cultured and checked for purity at Department of Microbiology and Biotechnology, and maintained in a slant of Blood agar base.

Preparation of stock solutions

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

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.

Determination of MIC of pure compounds

The MIC values were determined according a modified method (Bauer et al., 1966). The microplate (100 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 ul 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.

RESULTS AND DISCUSSION

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

Determination of antimicrobial activity

The antimicrobial activities of terpenoidal fractions from *A. leiocarpus* (Stem) and *T. avicennioides* (Root) were determined using some standard microorganisms (Table 2). The MIC of terpenoidal fractions were found to range from 0.213 to 4.05213 g/ml against *P. aeruginosa*, those of *S. aureus* are between 0.425 and 2.5 g/ml; while *E. coli* has the MIC of range 0.425 to 5.0 g/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	Saponin	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	0.425	0.425	0.213
Ta3	-	2.5	1.25
Ta4	NA	NA	NA
Ta5	NA	NA	NA
Ta6	-	0.625	0.625
Ta12	2.05	4.05	4.05
Alee	2.5	5.0	2.5
ALFA	NA	NA	NA
ALF3	-	2.5	2.5

- = Not done. NA = No activity

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 immunodeficient patients was susceptible to fractions Ta1, Ta3, Ta6, Ta12, Alee and ALF3.

It is a common practice among the traditional healers in Niger state to prepare an infusion of *A. leiocarpus* and *T. avicennioides* 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.

Conclusion

The present findings further confirm the efficacy 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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