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 antivirals 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., 2000; Sofowora, 1969; Mann et al., 2008). *Anogeissus* Adigun et al., 2001; Almagboul et al., 1988; Malcolm and leiocar-
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 Nigerian, 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 then dried 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 60HF254,366) 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 (F8-13), AlF2 (F14-20), AlF3 (F21-25), AlF4 (F26-30), AlF5 (F31-40), AlF6 (F41-45), AlF7 (F46-52), AlF8 (F53-60), AlF9 (F61-66) and AlF10 (F67-73) which 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 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). 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 sulfoxide (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.

RESULTS AND DISCUSSION

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

Determination of MIC of pure compounds

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 µg/ml against P. 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 1 to 7. Fractions 8, 9 and 10 were made up to 100 by 50 µl of broth. Well 8 is drug sterile, 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.

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.
Table 1. Phytochemical screening results of the pure compounds

<table>
<thead>
<tr>
<th>Pure Compound</th>
<th>Alkaloids</th>
<th>Anthraquinone</th>
<th>Carbohydrate</th>
<th>Flavonoid</th>
<th>Saponin</th>
<th>Steroids</th>
<th>Tannin</th>
<th>Terpenoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ta1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ta3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ta4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ta5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ta6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Ta12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alee</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ALFA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>ALF3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) - Present, (-) – Absent.

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

<table>
<thead>
<tr>
<th>Pure compound μg/ml</th>
<th>Staphylococcus aureus</th>
<th>Escherichia coli</th>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ta1</td>
<td>0.425</td>
<td>0.425</td>
<td>0.213</td>
</tr>
<tr>
<td>Ta3</td>
<td>2.5</td>
<td>NA</td>
<td>1.25</td>
</tr>
<tr>
<td>Ta4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ta5</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ta6</td>
<td>-</td>
<td>0.625</td>
<td>0.625</td>
</tr>
<tr>
<td>Ta12</td>
<td>2.05</td>
<td>4.05</td>
<td>4.05</td>
</tr>
<tr>
<td>Alee</td>
<td>2.5</td>
<td>5.0</td>
<td>2.5</td>
</tr>
<tr>
<td>ALFA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ALF3</td>
<td>-</td>
<td>2.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>

- = 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 treat, 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.

REFERENCES


