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Journal of Natural Sciences Research
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.6, 2014
www.iiste.org
IISTE

Antimicrobial Potentials of *Uvaria Afzelli* Scott-Elliot and *Tetracera Alnifolia* Willd Crude Extracts on Selected Human Pathogens: An *In – Vitro* Study

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Abstract
The antimicrobial activities of *Uvaria afzelii* Scott-Elliot root bark and *Tetracera alnjfolia* Willd leaf and root bark used in folklore medicine for the treatment of respiratory tract infections and superficial mycoses were studied. The chloroform and methanol extracts of the plants were screened against 3 Gram positive and 4 Gram-negative bacteria viz: *Staphylococcus aureus* UCH 2057, *Streptococcus pneumoniae* UCH 2034, *Bacillus subtillis* UCH 2033, *Pseudomonas aeruginosa* UCH 2058, *Klebsiella species* UCH 2046, *Proteus mirabilis* UCH 2055, and *Escherichia coli* UCH 2052. The activity of the crude extracts were also investigated on *Candida albicans* UCH STC 2036, *Aspergillus niger* PHM 1506, *Trichophyton mentagrophyte* ATCC 4808, *Trichophyton rubrum* ATCC 2894, *Epidermophyton floccosum* ATCC 110227 and *Microsporum canis* ATCC 11622. The agar diffusion and agar dilution methods were used for antimicrobial screening and determination of the minimum inhibitory concentrations (MIC) respectively. The methanol extracts of the plants at 10 mg/mL demonstrated the highest activity against most of the microorganisms tested. The M1C for the susceptible microorganisms ranged between 3.125 mg/mL to 50 mg/mL.
Phytochemical screening of the plant samples revealed the presence of tannins, saponins, cardenolides and alkaloids and the absence of anthraquinones. The test organisms were resistant to the drug controls but were susceptible to the extracts of the plants. The antimicrobial activities of the plants extracts on the microorganisms tested justify their use in folklore medicine for the treatment of respiratory tract infections and superficial mycoses.
Keywords: Antimicrobial activities, phytochemical screening, *Uvaria afzelii* Scott Elliot, *Tetracera alnifolia* Willd

1. Introduction

Plant kingdom holds many species of plants containing substances of medicinal value (Trease & Evans 2002). Large numbers of plants are constantly being screened for their possible pharmacological values such as their antimicrobial activities. Over the years, plants and plant materials have been used in the treatment of many diseases and infections. The plant *Uvaria afzelii* Scott Elliot (Annonaeceae) found mainly in tropical region (Graham & Bernard 1978) have been used traditionally in the treatment of bronchitis and cough (Burkill 1985) while *Tetracera alnifolia* Willd (Dilleniaceae), a pantropic plant has been reportedly used in the treatment of various diseases such as gastrointestinal diseases and infections like pulmonary and dermal infections including yaws (Walker & Sillans 1961).
There are different species of Uvaria and they are widely distributed in the tropics, Africa in particular (Akendengue *et al*, 2003; Graham &Bernard 1978). All the parts of the plant are fragrant and as such are used in the preparation of pomade in Ghana (Burkill 1985). *Uvaria afzelli* has been reported to have anti-parasitic activity (Okpekon *et al*, 2004). Other species of Uvaria have also found use in folklore medicine. This includes *U. doeringii*- the leaf decoction of which is taken for piles, palpitations and pains (Burkill 1985). *U. scabrida* is used in the treatment of insanity while *U. thomasii* is used in the form of a leaf decoction for catarrh and colic (Kerharo & Adam 1974). *U. tortilis* is used in the treatment of amenorrhoea (Borquet & Debray 1974).
Tetracera alnifolia belongs to the family Dillenaceae and is almost pantropical. *Tetracera alnifolia* and other species of Tetracera have been reported to contain chemical constituents such as flavonoids and coumarin derivatives which have been used in the treatment of various diseases and infections (Akendengue *et al*, 2003). The decoction of the leaf is given orally for the treatment of dysentery while the root macerate is used to treat urethral discharge (Walker & Sillans 1961). The roots of some Tetracera are used for yaws (Burkill 1985). The root of *T. affinis* is used for yaws. The plants lianous stems when macerated in its sap are administered for the treatment of leprosy (Kerharo & Adam 1962).
Since medicinal plants play a major role in the management of various ailments in rural communities of most developing nations, there is a need for scientific verification of their activities against some pathogenic bacteria and fungi. Currently, there is little evidence on the antimicrobial properties of *Uvaria afzelii* Scott Elliot and *Tetracera alnifolia* Willd. The aim of this study was to assay the extracts of these plants under investigation for their antimicrobial activities.

2. Materials and Methods

2.1 Plant collection and preparation of extracts

The root of *Uvaria afzelii* and the leaves and root of *Tetracera alnifolia* were collected. The samples were authenticated at the Herbarium of the Forestry Research Institute of Nigeria (FRIN) and were assigned voucher specimen numbers FHI 107510 and107511 respectively. The samples were air-dried, pulverized, weighed and subjected to exhaustive Soxhlet extraction with methanol. The extracts were concentrated and each concentrated extract was partitioned into *n*-hexane and chloroform. The different fractions were concentrated in-vacuo, dried, weighed and stored at 4°C. Extracts were reconstituted with 40% methanol to final concentrations of 10 mg/mL and 20 mg/mL for the screening.

2.2 Organisms

The organisms used for the study are shown in Table 1 and consisted of three Gram positive bacteria, 4 Gram negative bacteria, 4 dermatophytes, 1yeast and 1 mould. These were obtained from the University College Hospital (UCH), and Pharmaceutical Microbiology (Pharm. Micro.) laboratory (lab) of the University of Ibadan.

2.3 Phytochemical screening

The pulverized samples of the root bark of *Uvaria afzelii* and the leaves and root bark of *Tetracera alnifolia* were examined for the presence of alkaloids, anthraquinones, cardenolides, tannins and saponins using methods described by Harborne (1991).

2.4 Determination of Antimicrobial Activity

This was carried out using the agar well diffusion method (Adeniyi *et al,* 2006). A 0.2 mL of a 1:100 dilution of an overnight culture of each bacterium was used to seed sterile molten sensitivity test agar medium maintained at 45°C. The seeded agar was poured into sterile Petri dish, allowed to set and then dried in the incubator at 37°C for 20 mins. Sabouraud's dextrose agar was poured into Petri dish, allowed to set and then dried in the incubator. The dried SDA plates were carpeted with 0.2 mL of a 1:100 dilution of each fungal strain. A standard cork borer of 8mm diameter was used to cut equidistant wells in the agar. A 100 µL of each extract reconstituted with 40% methanol at 10 mg/mL and 20 mg/mL concentration was added to each well. Gentamycin at 10 µg/mL and griseofulvin at 50 µg/mL were the positive controls for bacteria and fungi respectively while 40% methanol was the negative control. The plates were incubated at 37°C for 24 hrs and at room temperature for 48°C for bacteria and fungi respectively.

Table 1: List of Microorganisms

MICROORGANISMS	CODE	TYPE	SOURCE	ANTIBIOGRAM
Staphylococcus aureus *S. aur*	UCH 2057	Gram positive cocci	UCH clinical isolate	Sens: AUG, AMX, CPF, CLOX, CEF, ERT, Res: GEN, CAF.
Escherichia colii *E. coli*	UCH 2052	Gram negative rod	UCH clinical isolate	Sen: GEN, SPF, CPF, CEF, Res: AMX, AUG, CFZ, PEF
Streptococcus pneumoniae *St. pneu*	UCH 2054	Gram positive rod	UCH clinical isolate	Sens: CPF, AMX, AUG, CEF: Res: GEN, COT, CLOX, ERT.
Proteus mirabilis *Pr. mir*	UCH 2055	Gram negative rod	UCH clinical isolate	Sens: GEN, OFL, CPF, AUG Res: AMX, COT
Pseudomonas aeruginosa *Ps. aeru*	UCH 2058	Gram negative rod	UCH clinical isolate	Sens: CPF, GEN Res: CFZ, CFX, CEF
Bacillus subtilis *B. sub*	UCH 2033	Gram positive rod	UCH clinical isolate	Sens: GEN, ERT, PEF Res: NFT
Klebsiella species *Kleb. spp*	UCH 2046	Gram negative rod	UCH clinical isolate	Sens: CRO Res: CPF, GEN, AUG, AMX, COT, PEF
Candida albicans *C. alb.*	UCH STC 2036	Yeast	UCH clinical isolate	
Trichophyton mentagrophtes *T. ment.*	ATCC 4808	Dermatophyte	Pharm. Micro. Lab. U.I	
Trichophyton rubrum *T. rub*	ATCC 2894	Dermatophyte	Pharm. Micro. Lab. U.I	
Epidermophyton floccosum *E. flo.*	ATCC 1102	Dermatophyte	Pharm. Micro. Lab. U.I	
Microsporum canis *M. can*	ATCC 1162	Dermatophyte	Pharm. Micro. Lab. U.I	
Aspergillus niger *A. niger*	PHM 1506	Mould	Pharm. Micro. Lab. U.I	

Key: AUG=Augmentin, AMX=Amoxicillin, CPF=Ciprofloxacin, CLOX=Cloxacillin, CEF=Cefuroxime, ERT=Erythromycin, GEN= Gentamycin, CAF= Cephalexin, SPF=Sparfloxacin, CFZ=Ceftriazone, PEF=Perfloxacin COT= Cotrimoxazole, NFT=Nitrofurantoin, CRO= Ceftazidime

2.5 Determination of Minimum Inhibitory Concentration (MIC)
The MIC of the methanol extracts of *Uvaria afzelii* Scott-Elliot and *Tetracera alnifolia* Willd were determined by the agar dilution technique as previously used (Rusell & Furr, 1972; Lajubutu *et al*, 1995). A 2 mL of the different concentrations of each extract was mixed with l8mL of molten agar (STA and SDA), poured into sterile Petri dish and allowed to set. The dried surface of the agar was streaked with overnight broth cultures of the bacteria, the yeast and the mould. Broth culture of a 48-hour grown dermatophytes were used. The plates were incubated at 37°C for 24 hrs and at room temperature for 48 hrs for bacteria and fungi respectively. The plates were examined for the presence or absence of growth and the lowest concentration preventing growth was taken as the MIC of the extract.

3. Results and Discussion

The choice of plants used in this study was based on their reported local uses in the treatment of various diseases and this study further elucidates on their antimicrobial activities. In this study the yield of the extracts was highest in methanol which contradicts the report of Cowan (1999) that ranked methanol second next to methylene dichloride in terms of yield in extraction of plant active components. The phytochemical screening of the plant samples revealed the presence of tannins, saponins, cardenolides and alkaloids and the absence of anthraquinones. Tables 2 and 3 show the results of the antimicrobial screening of the crude extracts. All extracts demonstrated different degrees of antimicrobial activity. The methanol extract of the plants demonstrated broad spectrum antimicrobial activity been active against both Gram positive and Gram-negative bacteria as well as the dermatophytes. The Gram-positive organisms (*Staphylococcus aureus* UCH 2057 *and Streptococcus pneumoniae* UCH 2054*)* and the dermatophytes (*T. rubrum* and *M. canis*) which were resistant to the drug controls (Gentamycin 10 µg/mL and Griseofulvin 50 µg /ml respectively) were susceptible to the methanol extracts. The Gram-negative organisms-*Pseudomonas aeruginosa* UCH 2058 and *Proteus mirabilis* UCH 2055 were susceptible to a lesser extent when compared with the drug control. The zone of inhibition produced by the plants extracts is an indication of the susceptibility of tested microorganisms to the plants. Also, the diameters of zones of inhibition were observed to vary from one organism to another and from one plant to another. These differences in the zones of inhibition observed is in accordance with the explanation of Prescott (2002) that the effect of an antimicrobial agent varies with the target species.
The MIC of the methanol extracts ranges from 3.125 mg/mL to 50 mg/mL (Table 4). The dermatophytes which are known to cause superficial infections (i.e. superficial mycoses) of the keratinized tissues (Brooks *et al*, 2007) are of medical importance. *Trichophyton rubrum* has been implicated in ringworm infections of the glabrous skin (*Tinea corporis*), nails (*Tinea unguium*), toe web (*Tinea pedis* or athlete's foot), the beard (*Tinea barbae*) and the groin (*Tinea cruris*). *Microsporum canis* causes ringworm infections of the scalp (*Tinea capitis*) and the glabrous skin (*Tinea corporis*) (Brooks *et al*, 2007). These infections are usually very difficult to treat because of the cellular structures of the dermatophytes which are closely related to that of humans hence, the activity demonstrated by the extracts against the dermatophytes in this study is noteworthy and can justify their use in the treatment of infections caused by these organisms. The plants extracts can also be used in the treatment of infections caused by the Gram positive bacteria-*Staphylococcus aureus* and *Streptococcus pneumonia*. *Staphylococcus aureus* has been implicated in boils, cabuncles, impertigo, pustles and wound infections, streptococcus pneumonia, otitis, sinusitis and other infections (Brooks *et al*, 2007).
The antimicrobial activity demonstrated by the methanol extracts of these plants is attributed to the presence of tannins and saponins which have been reported to possess antimicrobial activity (Trease & Evans 2002; Hou *et al,* 2000). The antimicrobial activities demonstrated by these plants therefore justify the ethnopharmacological claims.

4. Conclusion

The plants used in this study exerted antimicrobial activities on all tested microorganisms although at varying concentration due to differences in the concentration and test organisms. The use of herbs for the treatment of infections and diseases has over time proven to be effective as an alternative treatment; therefore, it should be promoted with scientific standardization. Further research on these plants will focus on identifying the most active phytochemical constituent responsible for the efficacy of extract used in this study.

Table 2: Antimicrobial Activity of *Uvaria Afzelii* (UA) Scott Elliot and *Tetracera alnifolia* (TA) Willd crude extracts at 10 mg/mL. Mean Diameter (mm) zone of inhibition + SEM

Extracts	**Organisms**												
	S. aur	***E. coli***	***B. sub***	***St. pneu***	***Ps. aeru***	***Kleb spp***	***Pr. Mir***	***A. niger***	***C. alb***	***T. ment***	***T. rub***	***E. flo***	***M. can***
cUArb	20 + 0.5	R	R	12 + 0.5	R	R	R	R	R	R	18 + 0.5	R	12+ 0.0
mUArb	15+ 1.0	R	R	16 + 0.0	R	R	12 + 1.0	R	R	R	16+ 1.5	R	20 + 0.0
cTAl	R	R	R	R	R	R	R	R	R	R	R	R	R
mTAl	16 + 1.0	R	R	R	R	R	15 + 1.0	R	R	R	20 + 0.5	R	15 + 1.0
cTArb	18 + 0.0	R	R	R	R	R	R	R	R	R	R	R	R
mTArb	15 + 1.5	R	R	20 + 1.0	R	R	14 + 0.5	R	R	R	R	R	18 + 0.5
Gent 10 μg/mL	R	25 + 0.0	35 + 0.0	R	42 + 0.0	27 + 0.5	32 + 0.5	NT	NT	NT	NT	NT	NT
Griseo 50 μg/mL	NT	NT	NT	NT	NT	NT	NT	R	R	R	R	R	R

Key: Diameter of cork borer = 8mm, R- Resistance, NT- Not Tested, cUArb - Chloroform extract of *Uvaria afzelii* root bark,
mUArb - Methanol extract of *Uvaria afzelii* root bark, cTAl - chloroform extract of *Tetracera alnifolia* leaf, mTAl - methanol extract of *Tetracera alnifolia* leaf, cTArb - chloroform extract of *Tetracera alnifolia* root bark, mTArb - methanol extract of *Tetracera alnifolia* root bark, Gent - Gentamycin, Griseo - Griseofulvin

Table 3: Antimicrobial Activity of *Uvaria Afzelii* (UA) Scott Elliot and *Tetracera alnifolia* (TA) Willd crude extracts at 20 mg/mL. Mean Diameter (mm) zone of inhibition + SEM

Extracts	**Organisms**												
	S. aur	***E. coli***	***B. sub***	***St. pneu***	***Ps. aeru***	***Kleb spp***	***Pr. mir***	***A. niger***	***C. alb.***	***T. ment***	***T. rub***	***E. flo***	***M. can***
cUArb	22 + 1.0	R	R	16 + 0.5	R	R	R	R	R	R	20 + 0.0	R	24 + 0.5
mUArb	20 + 0.5	R	R	20 + 0.0	18 + 1.0	R	15 + 1.0	R	R	R	20 + 0.5	R	22 + 1.5
cTAl	R	R	R	R	R	R	R	R	R	R	R	R	R
mTAl	18 + 0.5	R	R	20 + 1.5	16 + 0.5	R	R	R	R	R	24 + 0.5	R	22 + 0.0
cTArb	20 + 1.0	R	R	18 + 0.0	R	R	R	R	R	R	R	R	R
mTArb	20 + 0.5	R	R	22 + 0.0	12 + 0.5	R	16 + 0.5	R	R	R	22+ 0.5	R	20 + 0.5
Gent 10 μg/mL	R	25 + 0.0	35 + 0.0	R	42 + 0.0	27 + 0.5	32 + 0.5	NT	NT	NT	NT	NT	NT
Griseo 50 μg/mL	NT	NT	NT	NT	NT	NT	NT	R	R	R	R	R	R

Key: Diameter of cork borer = 8mm, R- Resistance, NT- Not Tested, cUArb - Chloroform extract of *Uvaria afzelii* root bark,
mUArb - Methanol extract of *Uvaria afzelii* root bark, cTAl - chloroform extract of *Tetracera alnifolia* leaf, mTAl - methanol extract of *Tetracera alnifolia* leaf, cTArb - chloroform extract of *Tetracera alnifolia* root bark, mTArb - methanol extract of *Tetracera alnifolia* root bark, Gent - Gentamycin, Griseo - Griseofulvin

Table 4: Minimum Inhibitory Concentration (MIC) of Methanol extracts of *Uvaria afzelii* (UA) Scott Elliot and *Tetracera alnifolia* (TA) Willd (mg/mL)

Organisms	mUArb	mTAl	mTArb	Gentamycin (μg/mL)	Griseofulvin (μg/mL)
S. aureus	12.5	12.5	12.5	R	NT
St. pneu	25.0	25.0	25.0	R	NT
Ps. aeru	50.0	50.0	50.0	10	NT
Pr. mir	50.0	50.0	50.0	10	NT
T. rubrum	6.25	12.5	3.125	NT	R
M. canis	6.25	12.5	3.125	NT	R

Key: mUArb - methanol extract of *Uvaria afzelii* root bark, mTAl - methanol extract of *Tetracer*a *alnifolia* leaf, mTArb - methanol extract of *Tetracera alnifolia* root bark

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