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Antimicrobial assessment of ethanolic extract of *Costus afer* Leaves

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ABSTRACT

The antibacterial effect of ethanolic leaf extract of Costus afer was evaluated on bacterial strains which included Streptococcus pneumoniae, Streptococcus pyogenes and Staphylococcus aureus, among the Gram-positive organisms and Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli among the Gram-negative, using Standard Microbiological Method [STM]. The in vitro antibacterial activity was performed by Agar-disc Diffusion Method [ADM]. The concentrations used were 150mg/ml, 100mg/ml and 50mg/ml. All the organisms tested varied in their percentage susceptible to the extract of the plant leaves at different concentrations, except Klebsiella pneumonia which was 100% resistant. The Gram-positive organisms were more sensitive to the plant extract than the Gram-negative organisms. The phytochemical analysis reveals the present of flavonoids, saponins, tannins, cardiac glycosides terpenes and phlobatanins. Cotus afer leaves can be used for treatment of disease associated with tested organisms excluding K. pneumonia.

INTRODUCTION

Some strains of clinically important pathogens have increased in antibiotic resistance, thus leading to the emergence of new bacterial strains that are multi-resistant [1]. Antibiotic resistance has become a global concern [2,3]. The relationship between human norms and plants is not limited to use of plant for food, shelter, and clothing alone but also include their use for ornamentation and health care. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens [3]. Chemotherapeutic agent act on a special target. Resistance arises when the target enzyme or the organelle is modified so that it is no longer susceptible to the drug. Resistance bacteria may either use an alternate pathway to bypass the sequence inhibited by the agent or increase the target metabolite. Some pathogens have plasma membrane translocases that expel drug[4] They are relatively nonspecific and can pump many different drugs. The occurrence of mutation on genes located on bacterial chromosomes may confer drug resistance on the cell. The occurrence of mutation to drug in bacterial population can be transferred to other cells by either conjugation, transduction or transformation mechanism [4].

Researchers are increasingly turning their attention to traditional medicine, looking for new leads to develop better drug against microbial infections. The increasing failure of chemotherapeutic agents and antibiotics resistance exhibited by pathogenic organisms has led to the screening of several medicinal plants for their potential antimicrobial activities. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products either as pure compound or as standardized plant extracts provide opportunities for new drug leads.

[5] reported that, the stem juice of *Costus afer* is used as a remedy for stomach ache, cough and rheumatic pains in the South-East rural communities in Nigeria. Information on the antimicrobial activities of many wetland plants in the tropics is necessary for utilization of such plants in the manufacture of drugs [6]. This study was designed to

undertake the phytochemical screening and in-vitro antimicrobial activity of *Costus afer* leaves against clinically important pathogens.

MATERIALS AND METHODS

Plant Materials:
Fresh leaves of *Costus afer* were collected from Ediene in Ikono Local Government Area of Akwa Ibom State, Nigeria. The leaves were identified by Taxonomist in the Department of Botany and Ecological studies, University of Uyo, Uyo and the voucher specimen, preserved and deposited in the herbarium.

Extraction and Analyses:
The leaves were sun dried and ground to powder using Mortar and pestle. One kilogram [1kg] of the leaf was weighed out and macerated with 70% ethanol for 72 hours. The extract was separated from the Mac by filtration. The filtrate was concentrated to dryness at 40^0C. The weight of the extract yielded was 30g.

Phytochemical Screening:
The extract was subjected to phytochemical screening using standard procedure as described by[7,8].

Preparation of Extract concentrations
The concentrations of the extract used were 50mg/ml, 100mg/ml and 150mg/ml were used for the screening. This was done by dilution method.

Sources and Maintenance of Organisms
They organisms were *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa.* were obtained from the University of Uyo Health Centre, Uyo [UUMC]. General Hospital, Anua [GHA]. May Fair Diagnostic Laboratory, Uyo [MFDL]. Front Line Laboratory, Uyo [FLL]. General Hospital Ikot, Ekpene [GHE]. They isolates were confirmed in Microbiology Department University of Uyo, Uyo. And were maintained on Mueller-Hinton Agar Medium [Oxoid, UK]. Eighteen to twenty-four [18-24 hour] old pure cultures were used.

Antimicrobial Bioassay
The antibacterial activity test of the extract was carried out using the agar-disc diffusion method [9]. The labeled plates were seeded with the test organisms. The concentrations of the plant extract used were: 150mg/ml, 100mg/ml and 50mg/ml. Sterile filter paper discs of 6mm in diameter were impregnated with various concentrations of the extract and placed on the seeded plates. These were incubated at 37^0C for 24 hours. After 24 hours of incubation, each plate was examined and the diameters of zones of inhibition were measured in millimeters [mm]. Using the Kirby Bauer classification method of inhibitory zone ≤12mm below was regarded as moderately sensitive/ resistant while from ≥15mm and above was considered highly sensitive.

RESULTS AND DISCUSSION

Nine hundred and ninety one [991] clinical bacterial isolates were collected from five different centers [Table 2]. Occurrence of different clinical bacteria were represented in [Table 3] from each centre, these may be as a result of present of infections caused by the organisms in the area. The frequency of clinical pathogens used were *Ps. aeruginosa* 58[5.9%], *S pneumonia 287* [29%] *S. aureus 319* [32%], *E. coli 156* [15.7%], *S. pyogenes 95* [9.6%] and *K. pneumonia 76* [7.6%] [Table 4]. The result of phytochemical screening reveals predominantly the present of tannins, flavonoids, phlobatanins and glycosides [Table 1]. The possession of these bioactive compound isolated from the leaves of this plant has revealed certain biological activities on some clinical pathogenic microorganism used [1].

The most susceptible organisms to the ethanolic extract of *Costus afer* were *S. aureus* [fig .2] followed by *S. pneumoniae* [fig. 1] and *E. coli* [fig. 3], while *P. aeruginosa* and *S. pyogenes* were susceptible at high concentration [150mg/ml and 100mg/ml] [fig 4 and 5] and, *K. pneumoniae* was not susceptible at all [fig.6]. The organisms varied in patterns of susceptibility but more sensitive at high concentration.

The results of this study have shown that the leaves of *Costus afer* extracts had varied antimicrobial activity against the organisms tested. This suggests the extract of this plant exhibited a broad spectrum activity since it was active against both the Gram-positive and the Gram-negative organisms tested. Its antimicrobial activity reveal high efficacy against Gram-positive organisms. This work conforms to the work of [10]. The high antimicrobial activity shown by this extract on the test organisms may be either due to the presence of alkaloids flavonoids, saponins,

tannins, steroids and cardiac glycosides [1,10]. The potentials for developing antimicrobials from plants seems rewarding as it will lead to the development of a phytomedicine against microbes. Antibiotics derived from plants have various therapeutic potentials as they can serve the purpose with lesser side effect that are often associated with synthetic antimicrobials [5,12]. Flavonoids are known for their anti-inflammatory and allergic effect coupled with their gastric mucus production. Flavonoids also possesses some antibacterial and antifungal properties [10].

Table: 1 Phytochemical screening of *Costus afer*

Test	Observation	Inference
Tannins	Blue green precipitate	+++
Saponin	Persistent frothing	+
Alkaloids	No precipitate/turbidity formed	–
Anthraquinone	No pink red or violet colouration At the lower phase.	–
Flavonoid	Orange colouration formed.	+++
Terpenes	A pink colouration at interphase	+++
Phlobatanin	Bulky precipitate with coloured Residue	+++
Glycosides		
a)Hydrolysis of glycosides	Brick red precipitate	++
b)Lieberman's	Colour change from violet to blue and to green	+++
c)Salkowski	Reddish brown colour at interphase	+++
d)Killer kiliani	Brown ring at the interphase	+++

Table 2: Centers and Number of Clinical Bacterial Isolates collected

Centers	Number of Isolates	% of Isolates
UUMC	250	25.2
GHA	201	20.3
MFDL	187	18.9
FLL	138	13.9
GHE	215	21.7
Total	991	100

Table 3: Clinical bacterial isolates collected from each center

Centers	Clinical isolates
UUMC	Abcdef
GHA	Abdf
MFDL	Bcef
FLL	Abce
GHE	Abef

Key: a=*S. pneumoniae*, b= *S. aureus*, c=*E. coli*, d=*Ps. aeruginosa*, e=*S. pyogenes*, f=*K. pneumoniae*

Table 4: Frequency and percentage of Clinical isolates

Clinical isolates	Frequency	Percentage
Streptococcus pneumonia	287	29
Staphylococcus aureus	319	32.2
Escherichia coli	156	15.7
Pseudomonas aeruginosa	58	5.9
Streptococcus pyogenes	95	9.6
Klebsiella pneumoniae	76	7.6
Total	991	100

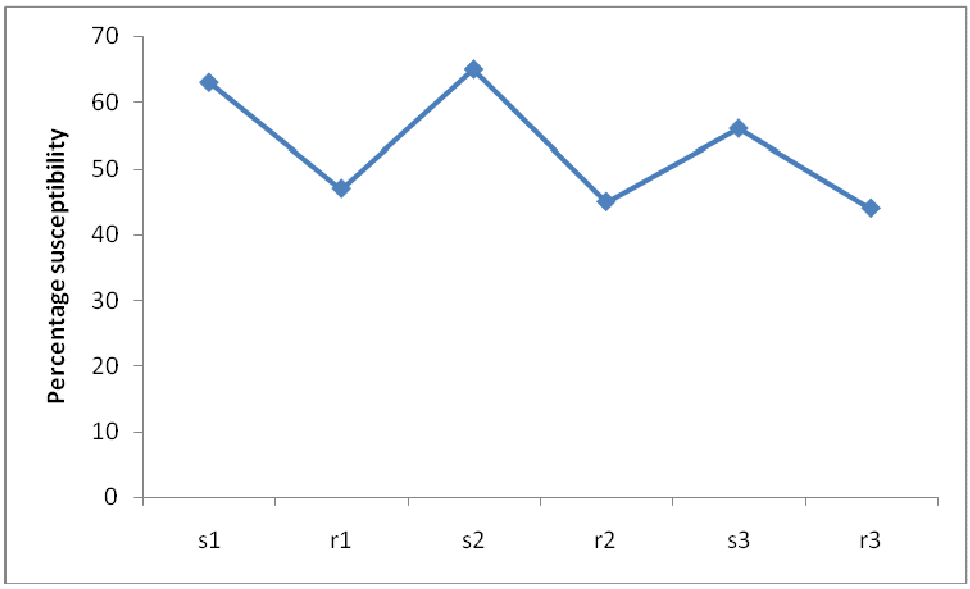

Fig.1 Susceptibility patterns of *S. pneumoniae* to the leaves extracts of *Cotus afer*

Key: s1= sensitive at concentration of 150mg/ml
r1=resistant at the concentration of 150mg/ml
s2=sensitivity at the concentration of 100mg/ml
r2=resistant at the concentration of 100mg/ml
s3=sensitivity at the concentration of 50mg/ml
r3=resistant at the concentration of 50mg/ml

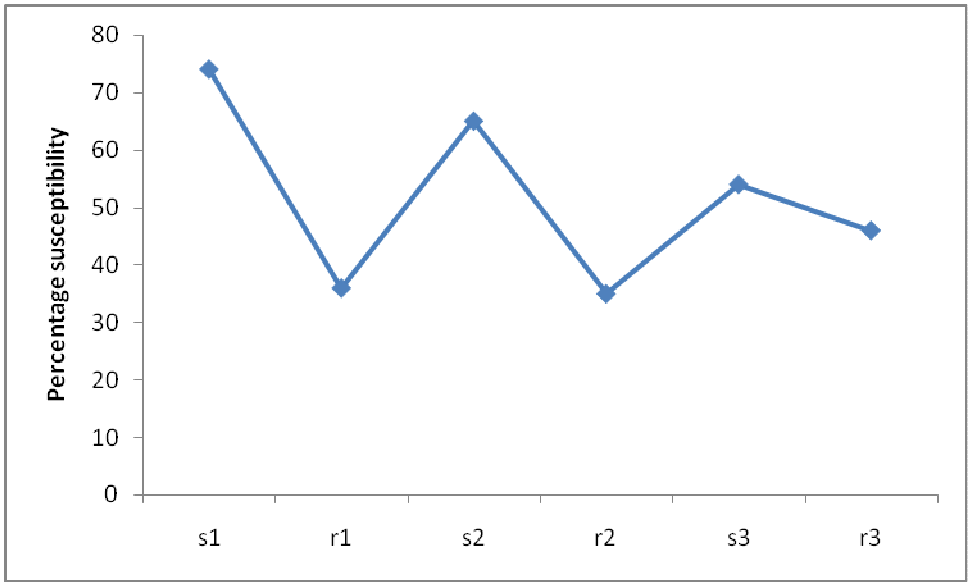

Fig.2 Susceptibility patterns of *S. aureus* to the leaves extracts of *Cotus afer*

Key: s1= sensitive at concentration of 150mg/ml
r1=resistant at the concentration of 150mg/ml
s2=sensitivity at the concentration of 100mg/ml
r2=resistant at the concentration of 100mg/ml
s3=sensitivity at the concentration of 50mg/ml
r3=resistant at the concentration of 50mg/ml

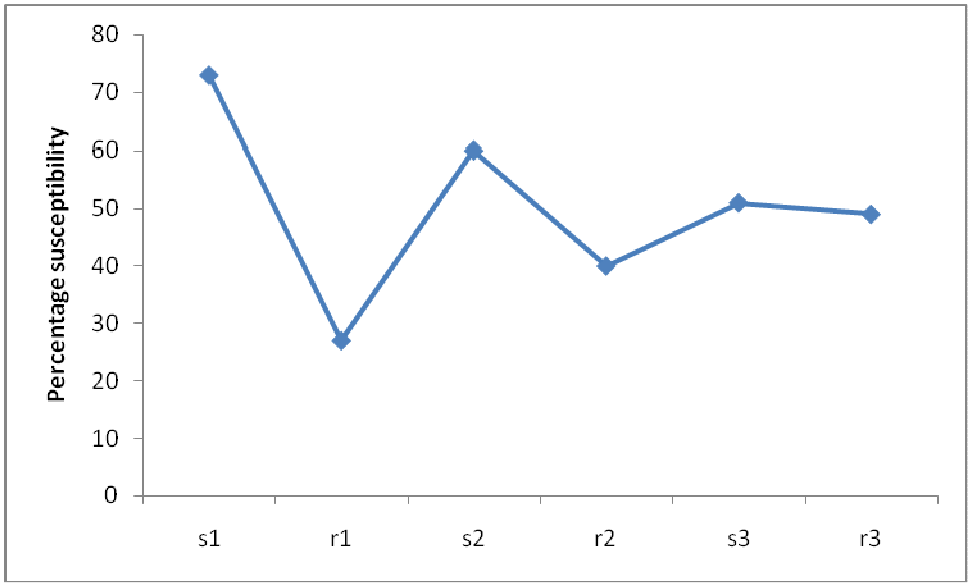

Fig.3 Susceptibility patterns of *E. coli* to the leaves extracts of *Cotus afer*

Key: s1= sensitive at concentration of 150mg/ml
r1=resistant at the concentration of 150mg/ml
s2=sensitivity at the concentration of 100mg/ml
r2=resistant at the concentration of 100mg/ml
s3=sensitivity at the concentration of 50mg/ml
r3=resistant at the concentration of 50mg/ml

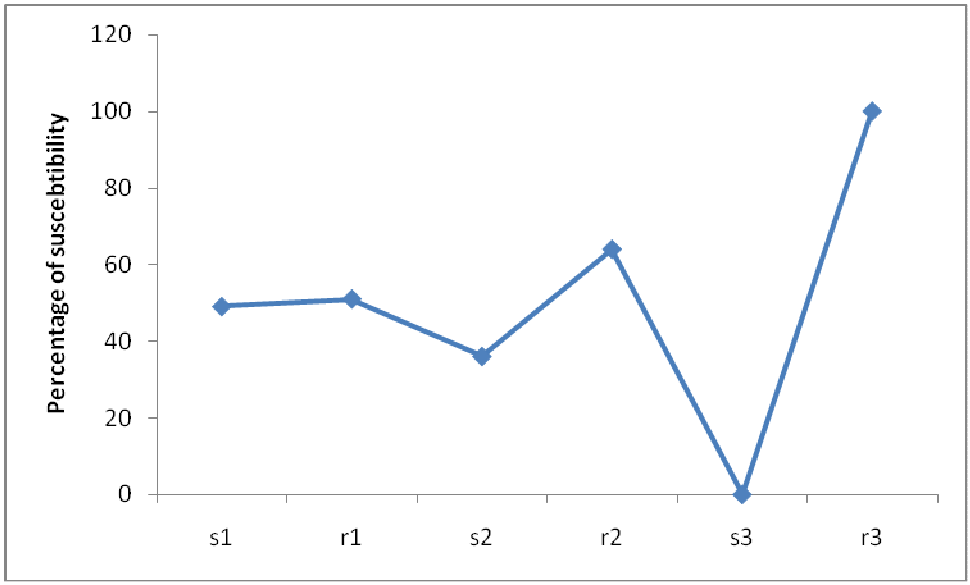

Fig.4 Susceptibility patterns of *P. aeruginosa* to the leaves extracts of *Cotus afer*

Key: s1= sensitive at concentration of 150mg/ml
r1=resistant at the concentration of 150mg/ml
s2=sensitivity at the concentration of 100mg/ml
r2=resistant at the concentration of 100mg/ml
s3=sensitivity at the concentration of 50mg/ml
r3=resistant at the concentration of 50mg/ml

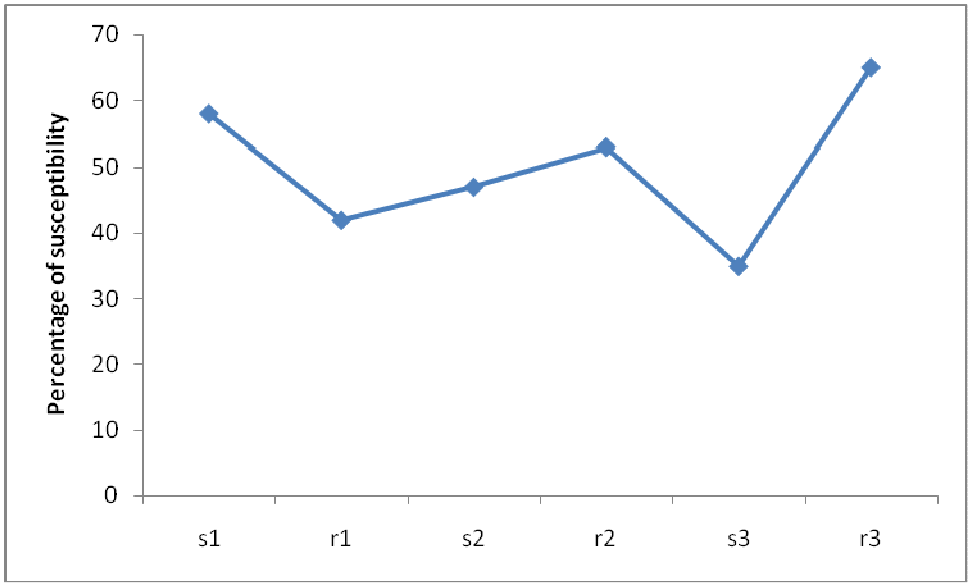

Fig.5 Susceptibility patterns of *S. pyogenes* to the leaves extracts of *Cotus afer*

Key: s1= sensitive at concentration of 150mg/ml
r1=resistant at the concentration of 150mg/ml
s2=sensitivity at the concentration of 100mg/ml
r2=resistant at the concentration of 100mg/ml
s3=sensitivity at the concentration of 50mg/ml
r3=resistant at the concentration of 50mg/ml

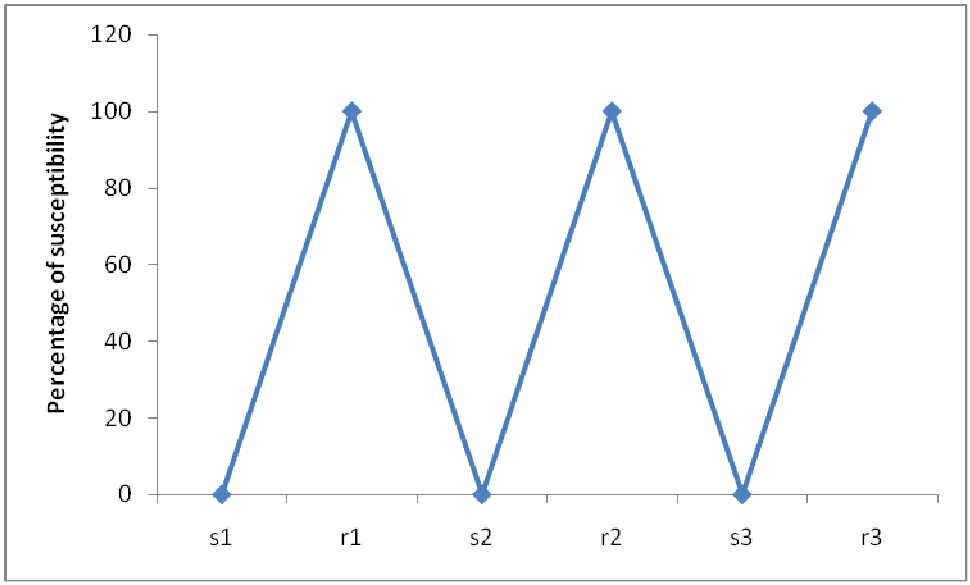

Fig.6 Susceptibility patterns of *K. pneumoniae* to the leaves extracts of *Cotus afer*

Key: s1= sensitive at concentration of 150mg/ml
r1=resistant at the concentration of 150mg/ml
s2=sensitivity at the concentration of 100mg/ml
r2=resistant at the concentration of 100mg/ml
s3=sensitivity at the concentration of 50mg/ml
r3=resistant at the concentration of 50mg/ml

The significant effect of this extract on some Gram-positive and Gram-negative organisms justifies the traditional use of this plant as a remedy for stomach ache, cough and rheumatic pains in some rural areas.

CONCLUSION

This study has shown the scientific basis for some of the therapeutic uses of this plant in traditional medicine. It also suggests ethno botanical measures should be considered when investigating antimicrobial properties of plants.

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