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Phytochemical Screening and Antibacterial Activity of Aqueous Extract of *Costus afer* Plant on Selected Multidrug Resistant Bacteria

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Abstract
Evidence abound on the use of various herbal plant in the treatment of infectious disease cause by MDR bacteria, owing to the present of bioactive compound. As such, this study was aimed at evaluating the phytochemical component and antibacterial activity of aqueous extract of Costus afer plant on selected multidrug resistant (MDR) E. coli, P. aeruginosa, S. typhi, Methicillin Resistant Staphylococcus aureus, Acinetobacter baumannii and K. pneumoniae. The leaf and stem of C. afer were collected from bush and garden respectively at Jamestown, in Eket L.G.A, Akwa Ibom state. The leaf and Stem of Costus afer were separately pulverized, processed and the filtrates were evaporated to dry at room temperature. Qualitative method was employed for phytochemical screening and Antibacterial activity was determined using Agar well Diffusion Method. The result of qualitative phytochemical analysis elucidate ample presence of Saponin, Tannins, Flavonoid, Alkaloids, Terpenes, Phlobataninin both stem and leaf except Anthraquinone identified only in stem extract. Aqueous leaf extract had excellent inhibitory activity on A. baumannii 17mm, E. coli 23mm, S. typhi 21mm, MRSA 30mm, K. pneumoniae 20mm and P. aeruginosa 23mm while aqueous stem had zones of inhibition of 22mm, 21mm, 18mm, 24mm, 18mm and 20mm recorded against A. baumannii, E. coli, S. typhi, MRSA, K. pneumoniae and P. aeruginosa respectively. There was no significant difference in zones of inhibition produce by the leaf and stem extract of Costus afer against the studied strain ($p \leq 0.05$). High inhibition zone of antibacterial activity reported in this study occur due to the presence of ample quantity of phytochemical compound and our findings justify the therapeutic use of Costus afer stem and leaf aqueous extract for the treatment of infections caused by studied MDR bacteria

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I. Introduction

Historically, plants have provided antimicrobials that produced successful results in the treatment of notable bacterial infections. The widespread incidence of antibiotic resistance displayed by clinical pathogen has increased over time. In order to curtail the scourge of AR bacteria, herbal plant research has been on the increase for their promising antibacterial activities. In light of the above fact, one of such plants with potential antibacterial activities is *Costus afer* also called bush cane or ginger lily. *Costus afer* is a member of the Costaceae Family (Edeoga and Okoli, 2000) which exist as unbranched and tall perennial herbaceous plant with creeping rhizome (Edeoga and Okoli, 2000). This herbal plant are geographically distributed in shady forest and riverbanks of Nigeria and other West African countries (Edeoga and Okoli, 2000). In Ibibio and Efik tribe it called "Mbritem", in Igbo tribe "Okpoo" or "Okpete", in Hausa "Kakizawa" and "Tete-egun" in Yoruba all in Nigeria (Anaga et al., 2004; Akpan et al., 2012). Apparently, herbal medicinal survey has reported the efficacy of *Costus afer* in particularly in treatment of cough, rheumatism, inflammation, cough, arthritis, hepatic disorders, miscarriages, helminthic, hemorrhoids and epileptic attack (Anaga et al., 2004; Akpan et al., 2012). Previous publisher data by Iwu, (2014) and Anaga et al., (2004) documented the use of *Costus afer* as diuretics, laxative and antidote that neutralize the effect of poison (Iwu, 2014; Anaga et al., 2004). According to Ethnobotanical survey of *Costus afer* plant the potent of the herb has been attributed to presence of phytomolecules such as saponins, flavonoids, tannins, terpenes, phlobatanins and cardiac glycosides (Akpan et al., 2012). The mechanism of action of phytomolecule in *Costus afer* are the major bioactive component with antimicrobial activity. Due to rising incidence of Multidrug resistant in bacteria and the spectrum or syndrome ofinfection ranging from mild to severe case cause by clinical pathogens such as *Klebsiella pnuemoniae, Pseudomonas aeruginosa, Escherichia coli*, Methicillin Resistant *Staphylococcus aureus* (MRSA), *Salmonella typhi*, *Acinetobacter baumannii* there is need to evaluate of *Costus afer* as a new drug lead against such MDR strain.

II. Methods

Collection and Processing *Costus afer*

The leaf and stem of *C. afer* were collected from bush and garden respectively at Jamestown, in Eket L.G.A, Akwa Ibom state. The leaf and Stem were authenticated by a taxonomist, Dr. Mbong E. O at Heritage polytechnic, Ikot Udoata Eket, Akwa Ibom state with voucher number CA002/HP/021.The leaf and Stem plant of *Costus afer* was washed and sun-dried for 3days and later pulverized using a sterile pestle and mortar (Aisha et al., 2016). The powder was stored in a sterile air-tight bottle for further use. Exactly 20g each for both weighed leave and stem powder were soaked in 200ml of water (20g each) for 24hours and thereafter filtered with muslin cloth according to Uwimbabazi et al. (2015). The filtrates were evaporated to dry at room temperature (Handa, 2013).

Confirmation of Clinical Strains

Pure colony of non-repeated and non-duplicated MDR clinical bacteria; *E. coli*, *P. aeruginosa*, *S. typhi*, Methicillin Resistant *Staphylococcus aureus*, *Acinetobacter baumannii* and *K. pneumoniae* were collected from Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA), Ebonyi State, Nigeria. The MDR clinical strains were confirmed using VITEK 2 System (bioMerieux, France) according to manufacturer's guideline.

Phytochemical Analysis

Qualitative method was employed for identification of phytochemical constituents such as Saponin, Tannins, Flavonoid, Alkaloids, Terpenes, Anthraquinone, Phlobatanin as described by Akpan et al. (2012) and Anyasor et al. (2010).

Antibacterial Assay of aqueous leaf and stem Extract of *Costus afer*

The Agar Well Diffusion Technique described by Katircioglu and Mercan, (2006) was used to screen for antibacterial activity of aqueous leaf and stem extract of *Costus afer*. Standard innoculum equivalent to 0.5 McFarland turbidity standard of the MDR clinical isolate was seeded on Mueller-Hinton agar plates. Five wells were bore on Solidified Meullar-Hinton agar plate using a sterile cork-borer of 6mm diameter. The plant part extract yield of 1.0 gram each from respective extraction solvent was reconstituted to 10^{-5} fold dilution using 70% Dimethylsulphoxide (DMSO) as a diluent. The extract diluted concentration of 100mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml were used to fill each agar well plate respectively. The inoculated plate were incubated at 37°C for 24-48 hours. Inhibition Zone was measured and recorded in millimeters.

Statistical Analysis

One-Way ANOVA was use to evaluate significant difference in zones of inhibition produce by the leaf and stem extract of *Costus afer* against the studied strain. $p \leq 0.05$ was considered to indicate significant difference.

III. Result and Discussion

Table 1: Phytochemical Analysis of *Costus afer*

	Leaf		Stem	
Test	**Observation**	**Inference**	**Observation**	**Inference**
Saponin	Persistent frothing	+++	frothing	++
Tannins	Blue green precipitate	+++	Blue green precipitate	+++
Flavonoid	Orange coloration formed	+++	Slight Orange coloration formed	++
Alkaloids	Precipitate formed	+++	precipitate formed	++
Terpenes	A pink colouration at interphase	+++	A pink colouration at interphase	+++
Anthraquinone	No pink red or violet coloration At the lower phase	-	Pink red or violet coloration at the lower phase	+
Phlobatanin	Bulky precipitate with colored residue	++	Bulky precipitate with slight colored residue	+

Key: - =Absent, **+**= Trace**, ++** = Moderately Present, **+++**= Present in High Concentration

Table 2: Inhibitory Zone Diameter of *Costus afer* against MDR Bacteria

Plant Part	**Extract Concentration**	***A. baumannii***	***E. coli***	***S. typhi***	**MRSA**	***K. pneumoniae***	***P. aeruginosa***	**p-*value****
Leaf								
	100mg/ml	17	23	21	30	20	23	
	50 mg/ml	12	20	15	23	15	18	.000059
	25 mg/ml	17	16	10	16	12	15	
	12.5 mg/ml	11	NI	NI	14	9	11	
	6.25 mg/ml	9	NI	NI	10	NI	7	
Stem								
	100mg/ml	22	21	18	24	18	20	
	50 mg/ml	17	19	13	20	15	16	.00001
	25 mg/ml	14	17	NI	18	13	14	
	12.5 mg/ml	10	12	NI	15	9	12	
	6.25 mg/ml	8	9	NI	12	NI	8	

Key: NI- No Inhibition, **MRSA-**Methicillin Resistant *Staphylococcus aureus*

The results of this study have shown that the aqueous leaf and stem of *Costus afer* extracts had concentration dependent antibacterial activity against test MDR clinical isolate. *Costus afer* leaf and Stem extracts had high inhibitory effect ranging from 12-30mm particularly at 100mg/ml and 50mg/ml against both Gram positive and Gram negative as well as fungal isolate. There was no significant difference in zones of inhibition produce by the leaf and stem extract of *Costus afer* against the studied strain ($p \leq 0.05$).Our findings substantiate result from other published data (Nna et al., 2019; John et al., 2018; Uchegbu et al., 2016) except for *A. baumannii* as this study is the first to report the antibacterial activity of *Costus afer* against the bacterial isolate. From the result section, low inhibitory activity was observed at 12.5mgml and 6.25mg/ml against few MDR clinical strain. This observation strongly indicate absence of ample quantity of the bioactive compound. Apparently, it could be postulated that bioassay are often concentration dependent, were increase in concentration is directly proportion the observed increase in zones of inhibition diameter.

From our result, stem and leaf extract produced high inhibitory effect of 24 and 30mm against MRSA while 21 and 23mm to *E. coli* at 100mg/ml. This data support earlier findings were Sidkey et al. (2013) who reported the effect of aqueous extract of *Costus afer* against MRSA and *E.coli* (Sidkey et al., 2013). Fraction I and fraction II obtained from column chromatography gave antimicrobial activity of 25.5 and 16.25mm, respectively against MRSA (Sidkey et al., 2013).

Our study revealed high antibacterial effect of *Costus afer* against *P. aeruginosa* and *K. pneumoniae* at different concentration as shown in the result section which is higher than report from other researchers; Uchegbu et al. (2016) reported zone of inhibition of 11.00mm *P. aeruginosa* and 10.00mm mm *K.pneumoniae* from stem extract of *Costus afer*. John et al. (2018) reported that ethanolic leaf extract of *Costus afer* has zone of inhibition of 9mm and 8mm for *P. aeruginosa* and *E. coli* at 150mg/l concentration. Nna et al. (2019) reported that stem ethyl acetate extract 14mm *E. coli* and 12 mm *P. aeruginosa*. Effiong and Obi (2018) reported that methanolic stem extract of *Costus afer* has zone of inhibition of 15.2mm and 17.1mm for *E. coli* respectively at 100mg/ml. This may be due to the known botanical fact that herbal plant from different locations may demonstrate different properties since they are grown on different soils and in varying climates and are exposed to different chemical and biological flora. However, the disparity may be attributed to the equipment and extraction methods wherein the active component of the leaf was better isolated.

Our study identified the presence of phytochemical compund such as anthraquinone and alkaloid which were not reported in earlier study (Akpanet al., 2012) and also in ample quantity in contrast to the findings of other researchers (Ukpabi et al., 2012; Anyasor et al., 2010). This study opined that the presences of such phytochemical compound (alkaloids and anthraquinone) indicate that *Costus afer* extract are more water soluble compare to other solvents found in existing literature.

This is an indication that the biological active compounds of *Costus afer* are more polar and as such are contained in the aqueous extraction of the leaves and stems. However, the study was able to produce larger zones of inhibition of 30.0mm.This observed antibacterial activity shown by this extract on the test organisms was clearly due to the presence of phytochemical compound such as terpenes, flavonoid, anthraquinone, tannins, saponins and phlobatanin, which demonstrate singly or synergistic mechanism of action toward the studied strain.

The result of this current study validate the judicious use of aqueous extract of *Costus afer* against MDR *E. coli*, *P. aeruginosa*, *S. typhi*, Methicillin Resistant *Staphylococcus aureus*, *A. baumannii* and *K. pneumoniae*. The spectrum of activity against the studied strain indicate that the plant could be harness for synthesis of broad spectrum antimicrobial agent. However, the toxicity of the assay extract require further evaluation.

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