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Phytochemical analysis and antifungal activity of *Costus lucanusianus* J. Braun & K. Schum aerial and rhizome crude extracts

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Abstract

The phytochemical screening, and *in vitro* antifungal activity of the inflorescence, leaf, stem and rhizome crude extracts of *Costus lucanusianus* J. Braun & K. Schum at concentrations 12.5-100 µg/mL were determined against 4 fungal strains; *Penicillium notatum, Candida albicans, Aspergillus niger* and *Rhizopus stolonifer* by Agar well diffusion method. Secondary metabolites accountable for the activity observed are saponin, tannin, flavonoid, phlobatannins, reducing sugar, anthraquinones, phenol, alkaloid, resin, glycoside and terpenoids. Reducing sugar was present in all the extracts. The rhizome solvent extracts displayed the highest antifungal activity amongst the organs extracts. The inflorescence, stem and rhizome aqueous extracts displayed no activity at all concentrations.

In this present study, the antifungal activity of *Costus lucanusianus* J. Braun & K.Schum aerial and rhizome extracts is reported. The activity shown by the plant organs justifies the use of the plant in the treatment of urinary tract infection and venereal diseases in ethnomedicine, and its usefulness in the preservation of food crops.

Keywords: Phytochemicals; Antifungal activity; Venereal diseases; *Costus lucanusianus* J. Braun & K. Schum

1. Introduction

Medicinal plants serves as an alternative therapy to antibiotics for many pathogenic microorganisms [1]. This is because they contain some important organic compounds (secondary metabolites), like carbohydrates, tannins, alkaloids, terpenes, flavonoids and steroids, which under *in vitro* conditions, are responsible for anti-microbial activities [2]. Medicinal plants and their herbal products usages in emerging countries, particularly in tropical Africa for curing ailments are still in practice [3][4].

Costus lucanusianus J. Braun & K. Schum (Costaceae) is an evergreen, perennial, rhizomatous, herbaceous and aromatic plant species with a thin stem that grows nearly vertically. The mature height of the plant is about 6-8 feet [5]. The bracts on the cone structure are open. Each bract covers one flower. The individual flowers are 1-1.5 inches across. Each has a thin, tissue-like texture. The stem grows about seven feet tall before it throws out a terminal inflorescence.

Inflorescence infusion of *C. lucanusianus* is used as a remedy for tachycardia and stomach problem. The leaf sap which is acidic is used in the treatment of eye problem and headache. The sap from the stem is used as a remedy for venereal diseases, urinary tract infection, urethral discharge, jaundice, miscarriage prevention and in Gabon, as an eye drop to control filariasis. The stem is also chewed to cure cough [5]. The ingestion of the rhizome decoction is also useful in the treatment of venereal, diarrhea, malaria and leprosy diseases [6]. It is extensively used in tropical Africa as a medicinal plant. The leaves have also been reported to be useful in the preservation of bitter kola. Pharmacological activities such as anti-inflammatory, antidiarrheal of the leaf aqueous extract [7], renoprotective, hepaprotective and

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antihyperglycemic of the leaf aqueous extract [8], tocolytic activity [9] have been reported. Nevertheless, little is known about its pharmacological activities and chemical components [8].

As a result of the medicinal importance of *C. lucanusianus* in the treatment of urinary tract infection and venereal disease, and its application in the preservation of food crops, this research is aimed to examine the phytochemical constituents and antifungal property of *C. lucanusianus* aerial and rhizome extracts.

2. Material and methods

2.1. Plant materials

Samples of inflorescence, leaf, rhizome and stem of *C. lucanusianus* were obtained fresh from Akobo Ibadan, Oyo state, South-west, Nigeria (at a geographical coordinate of 7° 25! 45.4" N 3° 56! 10.9" E; altitude 830 ft.) on 14th October 2015. The taxonomic identification of the plant materials was confirmed by a senior plant taxonomist, Mr L.T Soyewo of Forestry Research Institute of Nigeria (FRIN) herbarium, Ibadan. Voucher specimens (FH110048) were deposited at the FRIN herbarium.

2.2. Extraction of crude extracts

The collected inflorescence, leaf, stem and rhizome were washed thoroughly with water to remove soil particles, cut into pieces, air-dried for two weeks separately under shade. These were ground separately and kept in airtight containers until use.

Coarse inflorescence, leaf, stem and rhizome (50 g) each were cold macerated successively with hexane, ethyl acetate and methanol (200 mL each) for 72 hours. Extraction of each sample (50 g) with distilled water was also carried out separately for 48 hours. The filtration of the extracts was with Whatmann filter paper using Buchner funnel. Concentration of the filterates obtained was subsequently carried out by the use of rotary evaporator at 40°C under reduced pressure [2]. These extracts were put in a desiccator over anhydrous sodium sulphate until use. The weights and percentage yields (w/w) of the extracts were determined.

2.3. Qualitative Phytochemical Screening

The individual extracts were phytochemically screened qualitatively in order to determining the existence of secondary metabolites like tannins, alkaloids, flavonoid, saponin, triterpenes, resin, glycosides, reducing sugar, anthraquinone, phlobatannin and phenols using standard methods [10][11]. Stock solutions of each of the crude extracts were prepared by dissolving extracts (1 mg) in 10 mL of the individual mother solvents. The stock solutions obtained were subjected to phytochemical screening.

2.4. Antifungal activity

2.4.1. *Microbial Cultures*

Clinical strains of *Candida albicans,* and non-human pathogenic strains of *Penicillium notatum, Aspergillus niger* and *Rhizopus stolonifer* were supplied by the Pharmaceutical Microbiology Laboratory and Veterinary Medical Microbiology, University of Ibadan. Stock solution of each extract was prepared by dissolving 1 mg in 10 mL of their respective solvents to obtain a final concentration of 100 µg/mL. Four different concentrations of the stock solutions (12.5 µg/mL -100 µg/mL) were obtained by double-fold serial dilution.

2.4.2. *Antifungal assay: Preparation of inoculum*

Fungal strains taken from the stock were subcultured on to Sabour and dextrose agar (SDA). The mixture was incubated at 35°C for three days. The yeast spores obtained were suspended in sterile distilled water (5 mL) to obtain 10^5cells/mL. Dilution of the organisms to 1:100 was carried out and 0.2 mL of 1:100 dilution of the adjusted inoculum was taken and spread over the agar using a sterile spreader [12].

2.4.3. *Surface plate method*

The prepared sterile (SDA) (62 g/L) was left to solidify for 45 minutes in sterile plates in triplicate. The antifungal activity of the extracts was determined by using 30 µL of the each concentration (12.5 µg/mL -100 µg/mL). The experiment was performed in triplicate on (SDA) impregnated with fungal strains. Wells were made inside the set plates using 8 mm diameter sterile cork borer. Different concentrations of the extracts, and the controls (tioconazole 70%)

and mother liquor were poured into the wells and there was perfect diffusion into the agar for 120 minutes. Thereafter, the incubation of the plates uprightly for 48 hours at 28°C took place. The inhibition zone diameter (IZD) in mm was measured [13].

2.5. Statistical Analysis

All data were subjected to statistical analysis which was determined by making use of one way ANOVA. The differences between the data were considered significant at P≤0.05.

3. Results and discussion

Extracts yields (%) are presented in Table 1. The percentage extractive value of leaf hexane extract was the highest followed by the rhizome methanol extract. These low yields recorded in this present work corroborate the low yields reported for the extracts obtained from *Costus* genus [14][15].

Table 1 The colour and percentage yields of 16 extracts of *Costus lucanusianus* J. Braun & K. Schum

Extraction solvents	Plant materials	Colours	% Yields
Hexane	Inflorescence	Brown	0.20
	Leaf	Greenish black	3.00
	Stem	Light green	0.80
	Rhizome	Light brown	0.21
Ethyl acetate	Inflorescence	Brown	0.04
	Leaf	Greenish black	1.12
	Stem	Light green	0.15
	Rhizome	Light brown	0.07
Methanol	Inflorescence	Dark brown	0.57
	Leaf	Dark brown	0.65
	Stem	Brown	0.08
	Rhizome	Dark brown	1.20
Aqueous	Inflorescence	Dark brown	0.50
	Leaf	Dark brown	1.10
	Stem	Brown	0.06
	Rhizome	Dark brown	1.02

3.1. The phytochemical analyses results of *C. lucanusianus* solvent extracts are presented in Table 2.

Phytochemical screening of bioactive components of the inflorescence and rhizome of *C. lucanusianus* are reported for the first time.

The phytochemical screening results showed that *C. lucanusianus* is rich in phytochemicals amongst which were alkaloids, terpenoids, saponin etc (Table 2). The inflorescence methanol extract contained phlobatannin while anthraquinone was detected in the ethyl acetate. It was observed that the entire extracts obtained from the plant four organs contained reducing sugar.

Saponins, phlobatannin, reducing sugars, alkaloids and glycosides are present in all the aqueous extracts.

Table 2 Phytochemical analyses results of *C. lucanusianus* crude extracts

	Aqueous	Hexane	Ethyl acetate	Methanol	Aqueous	Hexane	Ethyl acetate	Methanol	Aqueous	Hexane	Ethyl acetate	Methanol	Aqueous	Hexane	Ethyl acetate	Methanol
Bioactive Components	**Inflorescence**				**Leaf**				**Stem**				**Rhizome**			
Saponin	+	-	+	+	+	+	+	+	+	-	+	+	+	+	-	+
Tannin	+	+	-	+	+	-	-	+	-	-	+	+	-	-	+	+
Flavonoids	-	-	+	-	-	-	+	-	-	-	+	+	-	-	-	+
Phlobatannis	+	-	-	+	+	-	-	-	+	-	-	-	+	-	-	-
Reducing sugar	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthraquinones	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenol	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-
Alkaloids	+	+	-	-	+	-	+	+	+	+	-	-	+	+	-	+
Resin	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	+
Glycosides	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+
Terpenoids	+	-	-	+	-	-	+	+	-	-	+	+	+	+	+	-

+ = Detected, - = Below detectable limit

Table 3A Antifungal activity results of crude extracts

Parts of plant used	Solvent extracts	*C. albicans*				*A. niger*			
	Concentration (µg/mL)	**100**	**50**	**25**	**12.5**	**100**	**50**	**25**	**12.5**
Inflorescence	Aqueous	-	-	-	-	-		-	-
	Hexane	15.3 ± 1.067	13.3 ±1.067	10	-	14.7 ± 1.067	12.7 ± 1.067	10	-
	Ethyl acetate	15. 3 ± 1.067	12.7 ±1.067	10	-	17.3 ± 1067	14.7 ± 1.067	12	10
	Methanol	13.3 ±1.067	10	-	-	13.3 ± 1.067	10	-	-
Leaf	Aqueous	18.7 ±1.067	16.7 ±1. 067	14	12	17.3 ± 1.067	14	12	10
	Hexane	-	-	-	-	-	-	-	-
	Ethyl acetate	17.3 ±1.067	14.7 ± 1.067	12.7 + 1.067	10	16.7 ± 1.067	14	12	10
	Methanol	16	14	12	10	18 ± 1.848	15.3 ±1.067	13.3 ± 1.067	10
	Aqueous	-	-	-	-	-	-	-	-
Stem	Hexane	14.7 ± 1.067	13.3 ± 1.06	10		14.7 ± 1.067	12	10	
	Ethyl acetate	20 ± 0.533	18.7 ± 1.067	16	14	18.7 ± 1.067	16.7± 1.067	14	12
	Methanol	15.33 ± 1.067	13-3 ± 1.067	10	-	15.33 ± 1.067	12	10	-
	Aqueous	-	-	-	-	-	-	-	-
Rhizome	Hexane	18.7 ± 1.067	16.7 ± 1. 067	15	14	19.3 ± 1.067	17.3 ± 1.067	15.3±1.067	13.3± 1.067
	Ethyl acetate	17.3 ± 1.067	14.7 ± 1.067	12	10	17.3 ± 1.067	15.3 ± 1.067	12.7 ± 1.067	10
	Methanol	18.7 ± 1.067	17.3 ± 1.067	14.7 ± 1.067	12	17.3± 1.067	14	12	10
	+ve standard Tiocanazole 70%			28		26			

**C. albicans= Candida albicans, A.niger= Aspergillus niger,*

Table 3B Antifungal activity results of crude extracts

Parts of plant used	Solvent extracts	*P. notatum*				*R. stolonifer*			
	Concentration (µg/mL)	**100**	**50**	**25**	**12.5**	**100**	**50**	**25**	**12.5**
Inflorescence	Aqueous	-	-	-	-	-	-	-	-
	Hexane	-	-	-	-	-	-		-
	Ethyl acetate	15.3± 1.067	12± 1.067	10	-	14	12	10	
	Methanol	15.3 ± 1.067	13.3 ±1.067	10	-	14.7 ± 1.067	12	10	
Leaf	Aqueous	-	-	-	-	-	-	-	
	Hexane	-	-	-	-	-	-	-	
	Ethyl acetate	14.7 ± 1.067	12	10	-	14	12	10	
	Methanol	14	12	10	-	16.7 ± 1.067	14	10	10
	Aqueous	-	-	-	-	-			
Stem	Hexane								
	Ethyl acetate	18.7 ± 1.067	16.3 ±0.533	14	12	20.7 ± 0.533	18	10	14.7 + 1.067
	Methanol	-	-	-	-	-			
	Aqueous	-	-	-	-	-			
Rhizome	Hexane								
	Ethyl acetate	19.0 ± 0.924	17.3 ± 0.533	15.0 ±0.924	12.7 ±1.067	18.7 ± 1.067	17.0 +0.924	15.0 +0.924	10
	Methanol	19.3 ± 1.067	17.3 ± 1.067	14	12	17.3 ± 1.067	14.7 +1.067	12	12.7 + 1.067
		16.7 ±1.067	14	12	10	16.7 + 1.067		14.7 + 1.067	
	+ve standard Tiocanazole70%	28				28			

P. notatum= Penicillium notatum, R. stolonifer= Rhizopus stolonifera

In this present study, the presence of saponin and absence of alkaloid in the stem methanol extract are reported. However, the existence of alkaloid and absence of saponins was recorded by [16]. Likewise, the present result of the leaf aqueous extract revealed the presence of alkaloids. However, the absence of alkaloids was reported by [15].

Climatic conditions, physiological state and geographical localization of the plants are part of the reasons that might be responsible for the varying chemical composition [17].

3.2. Antifungal activity

The antifungal activity results are shown in Table 3.

Rhizome organic solvent extracts showed activity against all the tested organisms at all concentrations while the leaf hexane extract was inactive against the four tested fungal. The leaf aqueous extract showed activity only against *C. albicans* (12.0 mm) and *A. niger* (10.0 mm) at the minimum concentration of 25 µg/mL while the aqueous extracts of the inflorescence, stem and rhizome organs displayed no antifungal activity against the tested fungal strains.

Amongst the 4 organs organic solvent extracts, the rhizome extracts displayed the highest antifungal activity especially against *C. albicans*. Statistically, there is no significant difference noticed between the activity of the various organic extracts of the rhizome organ.

The antimicrobial inactivity of *C. speciosus* rhizome methanol and aqueous extracts against *E. coli, S. aureus, K. pneumonia* and *P. aeruginosa* was documented by [18][19]. According to [18], *C. speciosus* rhizome hexane extract among others tested at 12.5 mg/mL displayed the highest antimicrobial property only against *B. subtilis* (12 mm) and *S. aureus* (15 mm), also the promising antifungal property of *C. speciosus* rhizome ethyl acetate fraction against *A. niger* and *C. albicans* was reported by [20].

The inflorescence, leaf and stem hexane extracts and the stem methanol extract of *C. lucanusianus* are resistant to *R. stolonifer* and *P. notatum*. The inactivity exhibited by the inflorescence, rhizome and stem aqueous extracts against all tested fungal may be because the active components are present in an inadequate amount in the concentrations of the extracts to be able to display activity [21]. Activity against other fungal strains not utilised in this present work may be shown by the extracts [22]. The antifungal activity shown by the plant extracts against *Penicillium notatum, Aspergillus niger* and *Rhizopus stolonifer* corroborates the usage of the plant in the preservation of bitter kola [23].

Figure 1 Matured plant of *Costus lucanusianus*

Photograph was taken in June 2016 at natural habitat, Akobo area, Ibadan North Local Government of Oyo State.

4. Conclusion

In this present study, the results of the phytochemical screenings of the extracts revealed the presence of an array of bioactive components. *C. lucanusianus* rhizome solvent extracts amongst other organs extracts displayed the highest activity against tested organisms. There was no antifungal activity displayed by the inflorescence, stem and rhizome aqueous extracts of *C. lucanusianus*. The antifungal property of inflorescence, leaf, stem and rhizome solvent extracts of *C. lucanusianus* against *C. albicans* could thereby justify the use of the plant in traditional medicine in the treatment of urinary tract infection and venereal diseases. Likewisely, the other activity shown against the non-human pathogens also supported the use of the plant in the preservation of food crops locally.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declares that there is no competing interest.

Statement of informed consent

The authors give their consent for the publication.

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