Phytochemical, antimicrobial and proximate analysis of *Costus spectabilis* grown in different soil types

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Abstract The search for medicinal plants with novel properties for therapeutical potentials is a continuum. The preliminary phytochemical analysis, antimicrobial properties and proximate contents of *Costus spectabilis* leaf and root harvested from different soil types were studied following standard protocols. The ethyl ethanoate extracts were assayed against six microbial cultures of *Staphylococcus aureus* (ATCC 29523), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633), *Aspergillus niger, Candida albicans*, and *Aspergillus clavatus* using the agar well diffusion methods. The phytochemical analysis revealed the presence of alkaloids, saponin, phenol, flavonoids and anthraquinone in all samples at varying concentrations. Terpenes and phlobatannins were not observed in any extract. Phenolic compound was observed to have the highest quantity of 6.18±0.00 mg/g from the leaf extract of the plant grown in loamy soil. Tannin and flavonoids were only detected in very insignificant quantities. The extracts showed significant inhibitory potential against the bacterial strains. The highest zone of inhibition was recorded as 33.02±1.45 mm against *B. subtilis* in the leaf extracts of the plant grown in clay soil. The leaf extracts from the plant obtained from clay soil showed fungicidal activity against the *Aspergillus* species, while the other extracts only showed fungistatic effects against all sampled fungal species. The proximate content analysis revealed that carbohydrates and protein are the dominant nutrients in the leaf of *C. spectabilis. Costus spectabilis* is a plant that can be exploited for its medicinal, nutritional and economic values.

Keywords: Antimicrobial; *Costus spectabilis*; Inhibition zone; Phytochemical; Proximate; Soil

Introduction

Costus spectabilis (Fenzl) Schumann is a perennial rhizomatous herb which belongs to the family Costaceae (Pacific bulb society, 2014). The term "spectabilis" is derived from the word "spectacular" and refers to the beauty of flowering plants (Specht and Stevenson, 2006). The plant is native to tropical Africa including Nigeria and it is distinguished with four large leaves which spread out horizontally to the ground to form a basal rosette. It is reported to produce yellow flowers at the centre where the inflorescence is located (Maas-van de Kamar et al., 2016).

Plants have been playing an important role in the treatment of several ailments since ancient time. *Costus spectabilis* is particularly used to treat cataract in humans (Shehu et al., 2019 and Shehu et al., 2022). According to Keïta et al. (2019) methanolic leaf extract of *C. spectabilis* has shown therapeutic properties for inflammation, arthritis, rheumatism, maternal and neonatal infections.

Medicinal plants are rich in a wide variety of secondary metabolites such as tannins, saponins, alkaloids, phenolic compounds, and flavonoids (Ebabhi et al., 2019 and Asekunowo et al., 2022). Therefore, phytochemicals found in medicinal plants are crucial in the development of novel healthcare and pharmaceutical products (Altemimi et al., 2017). Also, the availability of nutrients in different soil types, and the process of soil management play various important roles in the

growth, yield and quality of different plant species (Ohshiro et al., 2016). This study therefore is aimed at evaluating the phytochemical, antimicrobial and proximate contents of the ethyl ethanoate extract of the leaves and roots of *Costus spectabilis* grown in different soil types.

Materials and Methods

Collection, Preparation and Identification of Plant Materials

Leaves and roots were collected from freshly harvested rhizomes of *Costus spectabilis* obtained from Dreamscape Garden Resource, Akoka – Yaba, Lagos State, Nigeria. Potted planting of this species began in March 2020 using different soil types such as sandy, clay, loamy, silt, and mixed soil (ratio 2:1, clay and sandy) and it ended in July 2020 (Figure 1). A duplicate of each soil sample was used for this study. Watering of the plants was done daily and periodic weeding was observed. *Costus spectabilis* leaves and roots were harvested in good conditions. The plants were properly cleansed in tap water. They were then stretched out and dried for two weeks at ambient temperature (23 ± 2 ℃). The dried leaves and roots were pulverized, weighed and packed in different air-tight bottled containers and well labelled showing the different soil types. Plant material was identified at the University of Lagos Herbarium with a voucher number LUH8780.

Preparation of Plant Extracts

From the pulverized plant materials, 100 g of leaves and 50.5 g of roots were dissolved in 400 ml and 170 mL of ethyl ethanoate respectively at room temperature while shaking on an electric shaker for three days. Each was filtered using double layers of muslin cloth, centrifuged at 9000 rpm for 10 min. The supernatant was sifted through Whatman filter paper No 1 and the filtrate were then evaporated and dried at 40 ℃ in rotary evaporator. The extracts yields were weighed, labelled, and kept in glass bottles in the refrigerator at 4 ℃ until further use. Yield percentages were calculated using the formula of Extract yield % = R/S × 100 (where R; weight of plant extracts and S; weight of plant sample).

Preliminary Phytochemical Analysis

Phytochemical screening was carried out on the leaf and root ethyl ethanoate extracts of *Costus spectabilis* using standard procedures (Dhani, 2012; Hossain et al., 2013; Auwal et al., 2014).

Collection and Preparation of Inoculum

A total of six microbial cultures collected from the Microbiology Unit, Lagos University Teaching Hospital (LUTH), Lagos, Nigeria was used in this study. These were three bacterial strains - *Staphylococcus aureus* (ATCC 29523), *Pseudomonas aeruginosa* (ATCC 27853) and *Bacillus subtilis* (ATCC 6633) as well as three species of fungi- *Aspergillus niger, Candida albicans,* and *Aspergillus clavatus.* The fungal species were sub-cultured on freshly prepared commercially produced potato dextrose agar (PDA) plates which was prepared according to manufacturer's specification (Oxoid, Basingstoke, England). The pure cultures were identified following standard conventional protocols described by Vashishta and Sinha, (2014) while the bacteria strains were confirmed using the techniques described by Fawole and Oso (2004).

Inoculum Standardization

The pure cultures of the bacterial strains were obtained by inoculating freshly prepared Mueller Hinton agar plates and plates were incubated at 37 ℃ for 24 hours. Inoculum from each plate was then aseptically transferred into 20 mL of freshly prepared nutrient broth and incubated between 2 to 3 hours at 37 ℃ adjusting the turbidity to 0.5 McFarland standards giving a final inoculum of 2x10^6 CFU/mL. One capsule each of Chloramphenicol (500 mg) and Azole (500 mg) (Acichem laboratories and Fischer Scientific) were used respectively in the growth media to dissuade bacterial and fungal growth in the media.

Antimicrobial Assay of Plant Extracts

Antimicrobial assay of extracts of the plants was performed by agar well diffusion method as described by Manandhar *et al* (2019). Mueller Hinton agar plates and Potato dextrose agar plates were swabbed using sterile cotton swabs with 8

hours old broth cultures of respective bacterial and fungal test organisms. The inoculums were allowed to dry for 5 minutes. Holes of 6 mm were bored in each of the inoculated media plates using a sterile cork borer. A stock solution of each part of the plant extract was prepared at a concentration of 1g in 5 mL ethyl ethanoate. Each well was filled with 25 µL extracts separately from the leaf and root extracts. Positive control (Ciprofloxacin 5 µg) for bacteria and Nystatin 5µg/ml for fungal isolates and negative/solvent control (DMSO) respectively were also set up. The experiments were done in triplicate. Each was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37 ℃. After the incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm using a calibrated rule.

Determination of Minimal Inhibitory Concentration of the Plant Extracts

The broth microdilution method was used to determine the MIC according to Clinical and Laboratory Standards Institute (CLSI) guideline (2018). Two-fold serial dilutions of extracts were prepared directly in test tubes containing Mueller Hinton broth to obtain various concentrations in the range of 10, 5, 2.5, 1.25 and 0.625 ug/mL. Then an equal volume of bacterial inoculum was added to give a final concentration of 5×10^5 CFU/mL in each test tubes. The tubes were covered with a sterile sealer and incubated for 24 hours at 37 ℃. After incubation, growth was examined and data were taken. The MIC was considered as the lowest concentration of the extract that completely inhibits bacterial growth (CLSI, 2018). The experiment was repeated thrice for authentication of the data.

Procedure for Proximate Analysis

After bringing the samples to uniform size by obtaining the equal amount of each sample, they were analyzed for moisture, protein, fat, ash, fiber and nitrogen free extract by the methods of AOAC (2012).

Statistical Analysis

The data collected were subjected to single factor Analysis of Variance (ANOVA) using a statistical package Microsoft Excel for Data Analysis. Mean values that were significant were separated using Least significant difference (LSD) and the ANOVA test was carried out at 0.05 level of significance.

Results

Yield Percentage of the Crude Extract of Costus spectabilis Leaf and Root

The weights of the pulverized leaves were taken to be 113.30 g, 123.98 g, 151.20 g, 121.65 g and 132.08 g for sandy, clay, loamy, silt and mixed soil while the pulverized roots were taken to 50.6 g, 50.66 g, 69.01 g, 50.65 g and 51.3 g for sandy soil, clay soil, loamy soil, silt soil and mixed soil respectively.

The various extract yield from the different soil samples showed slight variations for the leaf and root of *C. spectabilis*. The leaf and root extracts of plants grown in loamy soil showed the highest yield of 12.01 % and 6.04 % respectively. The leaf extracts had more percentage yield than the root extracts for plant samples obtained from all soil samples. This is displayed in Table 1 while Figure 1 shows the various shoot aerial of the plant as grown in the different soil types.

Table 1: Yield of the Crude Extracts of *Costus spectabilis* Leaf and Root Grown in Different Soil

Soil types	**% Extract yield (leaf)**	**% Extract yield (root)**
Sandy	9.98	4.86
Clay	10.14	5.16
Loamy	12.01	6.04
Silt	10.01	5.00
Soil mixture	11.03	5.22

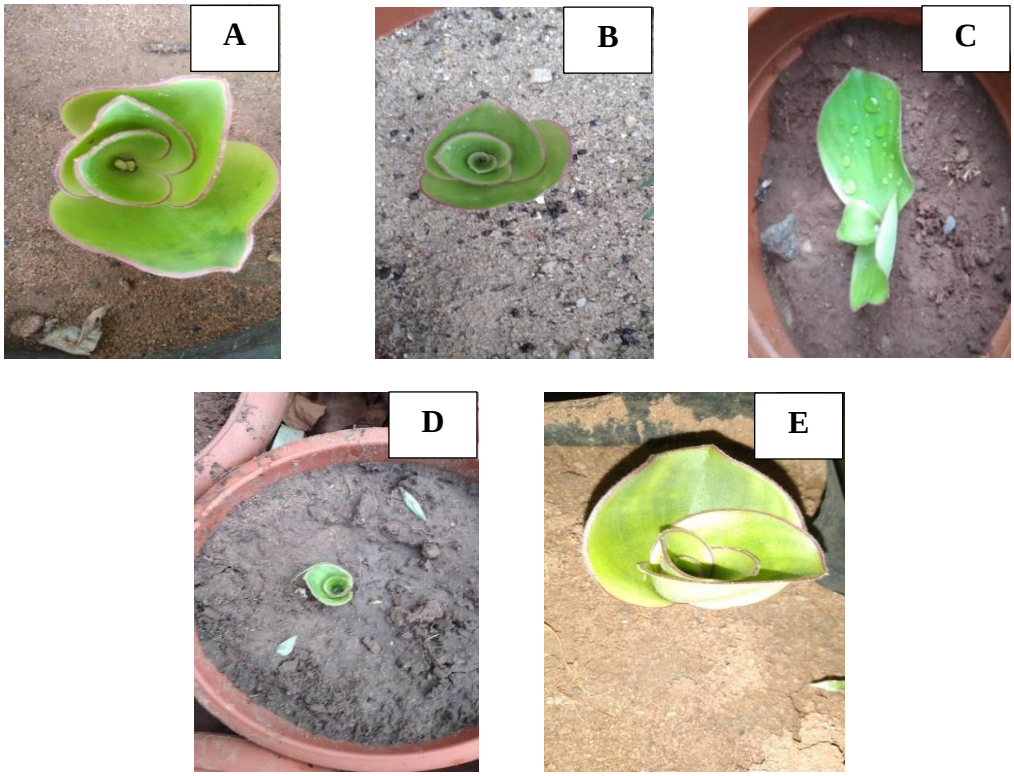

Figure 1: *Costus spectabilis* grown in different soil types; A: sandy soil, B: silt soil, C: soil mixture, D: loamy; E: clay soil.

Qualitative Phytochemical Screening

Preliminary phytochemical screening was carried out on the leaf and root extracts of *Costus spectabilis* harvested from different soil types which include clay, sandy, loamy, silt and mixed soil. The results showed the presence of alkaloids, saponin, phenol, flavonoids and anthraquinone in all samples. Phlobatannins and terpenes were not detected in any sample. Glycosides were only detected in the leaf extracts obtained from the plants grown in clay, sandy and mixed soil while steroids were detected in the leaf extracts of samples grown in clay and loamy soil. The qualitative phytochemical result is presented in Table 2.

Table 2: Qualitative analysis of the ethyl ethanoate leaf and root extracts of C. *spectabilis*

Soil type	**Silt**		**Clay**		**Sandy**		**Loamy**		**Mixture**	
Plant part	**Leaf**	**Root**	**Leaf**	**Root**	**Leaf**	**Root**	**Leaf**	**Root**	**Leaf**	**Root**
Phytochemical assayed										
Alkaloids	+	+	+	+	+	+	+	+	+	+
Saponin	+	+	+	+	+	+	+	+	+	+
Phenol	+	+	+	+	+	+	+	+	+	+
Phlobatannins	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+	+	+	+
Terpenes	-	-	-	-	-	-	-	-	-	-
Tannins	+	+	+	+	+	+	+	+	-	+
Glycosides	-	-	+	-	+	-	-	-	+	-
Steroids	-	-	+	-	+	+	+	-	-	-
Anthraquinone	+	+	+	+	+	+	+	+	+	+

Note: + = present; - = absent (ND)

Quantitative Phytochemical Screening

The quantitative analysis results of the ethyl ethanoate extracts of *C. spectabilis* leaf and root grown on different soil types is depicted in Table 3. It was observed that alkaloids were the highest (2.07 ± 0.03 %) in the leaf extracts of plant samples grown in clay soil. The extract of leaves harvested from the loamy soil showed the highest quantity of saponin at 0.33 ± 0.0 g/g while minute quantities of flavonoids and tannin were observed in all samples grown in the different soil types. Phenol was observed as the most prominent phytochemical in the plant samples as all extracts revealed a quantity that was >1.00 %. Glycoside content was highest at 19.46 ± 0.03 % in the extract of leaves grown on clay soil.

Table 3: Quantitative analysis of ethyl ethanoate leaf and root extracts of C*ostus spectabilis*

Soil type	**Silt**		**Clay**		**Sandy**		**Loamy**		**Mixture**	
Plant part	**Leaf**	**Root**	**Leaf**	**Root**	**Leaf**	**Root**	**Leaf**	**Root**	**Leaf**	**Root**
Phytochemical assayed										
Alkaloids (%)	1.53± 0.04	0.62± 0.00	**2.07± 0.03**	1.11± 0.00	0.99± 0.00	0.36± 0.00	1.96± 0.01	0.61± 0.01	1.48± 0.00	0.95 ±0.01
Saponin (g/g)	0.21± 0.00	0.14± 0.00	0.24± 0.00	0.13± 0.00	0.16± 0.00	0.08± 0.00	**0.33± 0.00**	0.12± 0.00	0.13± 0.00	0.21 ±0.01
Phenol (mg/g)	5.87± 0.01	3.99± 0.00	4.72± 0.01	4.86± 0.00	4.62± 0.01	4.54± 0.00	**6.18± 0.00**	5.29± 0.01	3.37± 0.00	3.99 ±0.01
Flavonoids (mg/g)	0.02± 0.00	0.03± 0.02	0.02± 0.00	0.01± 0.00	0.01± 0.00	0.02± 0.02	0.02± 0.00	0.03± 0.02	0.01± 0.00	0.02 ±0.00
Tannins (%)	0.03± 0.00	0.01± 0.00	0.03± 0.00	0.01± 0.00	0.07± 0.00	0.01± 0.01	**0.09± 0.00**	0.11± 0.01	ND	0.14 ±0.00
Glycosides (%)	ND	ND	**19.46 ±0.03**	ND	7.25± 0.00	ND	ND	ND	6.87± 0.03	ND
Anthraquinone (%)	0.09± 0.01	0.02± 0.00	**11.67 ±0.00**	0.05± 0.01	4.36± 0.00	0.04± 0.00	0.06± 0.01	0.06± 0.00	4.43± 0.00	9.86 ±0.01

* Values are expressed as mean ± standard error (bolded values represent highest values obtained per sample)

Antimicrobial Analysis

The antimicrobial potentials of the leaf and root extracts of *Costus spectabilis* harvested from different soil types were assayed on six microbes of *Staphylococcus aureus* (ATCC 29523), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (ATCC 6633), *Aspergillus niger*, *Candida albicans*, and *Aspergillus clavatus.* The results are shown in Figures 2 and 3, respectively, for the leaf and root extracts. It was observed that the leaf extracts of *C. spectabilis* grown on all soil types showed high potency against the test organisms. The highest zone of inhibition in the leaf extracts was 33.02±1.45 mm against *B. subtilis* from the plant grown in clay soil while the highest zone of inhibition in the root extracts was 32.05±0.14 mm against *B. subtilis* from the plant grown in mixed soil. All the leaves extracts except for the sample obtained from the plants grown in the mixed soil inhibited the bacterial growth. It was observed that the root extracts of the plant grown in the sandy and clay soil samples had no effect on the bacterial isolates but showed inhibitory potency on the fungal isolates while the root extracts of plant obtained from the mixed soil showed inhibitory effect only on the bacterial isolates.

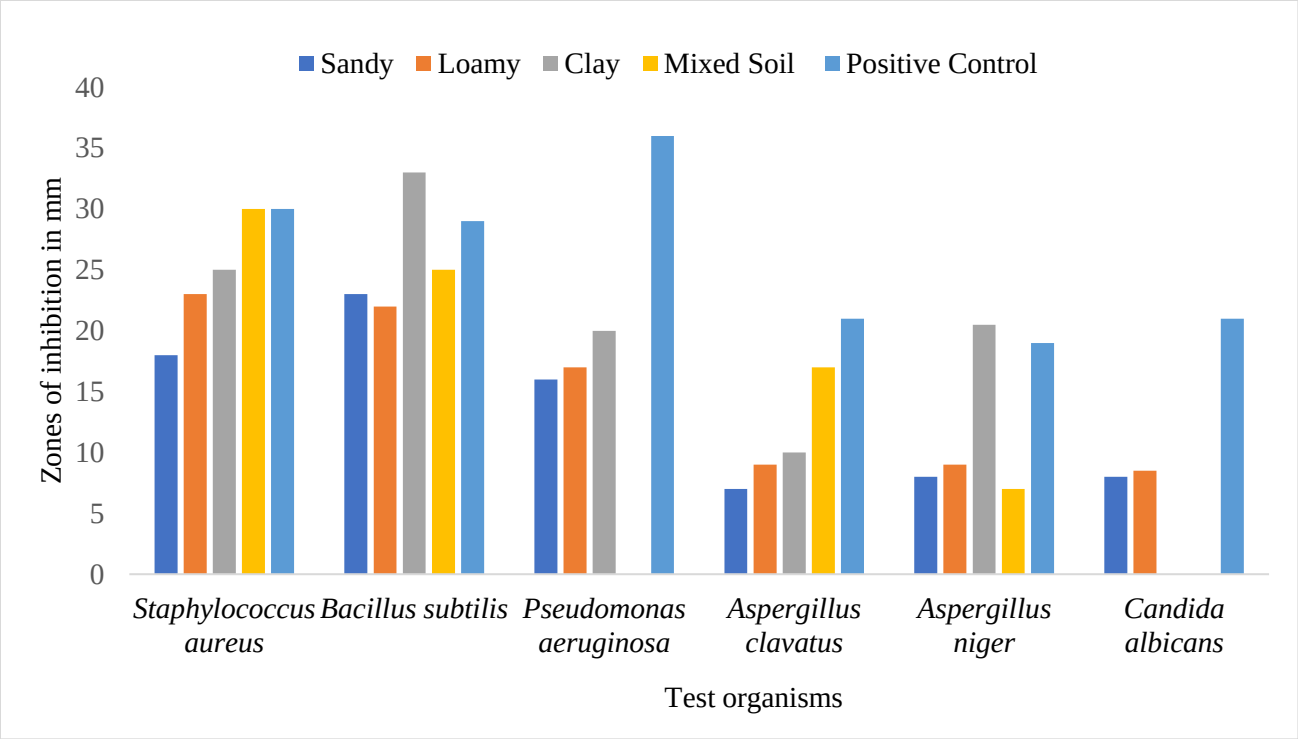

Figure 2: Zones of inhibition by the test organisms in the leaf extracts of *C. spectabilis* grown in different soil types.

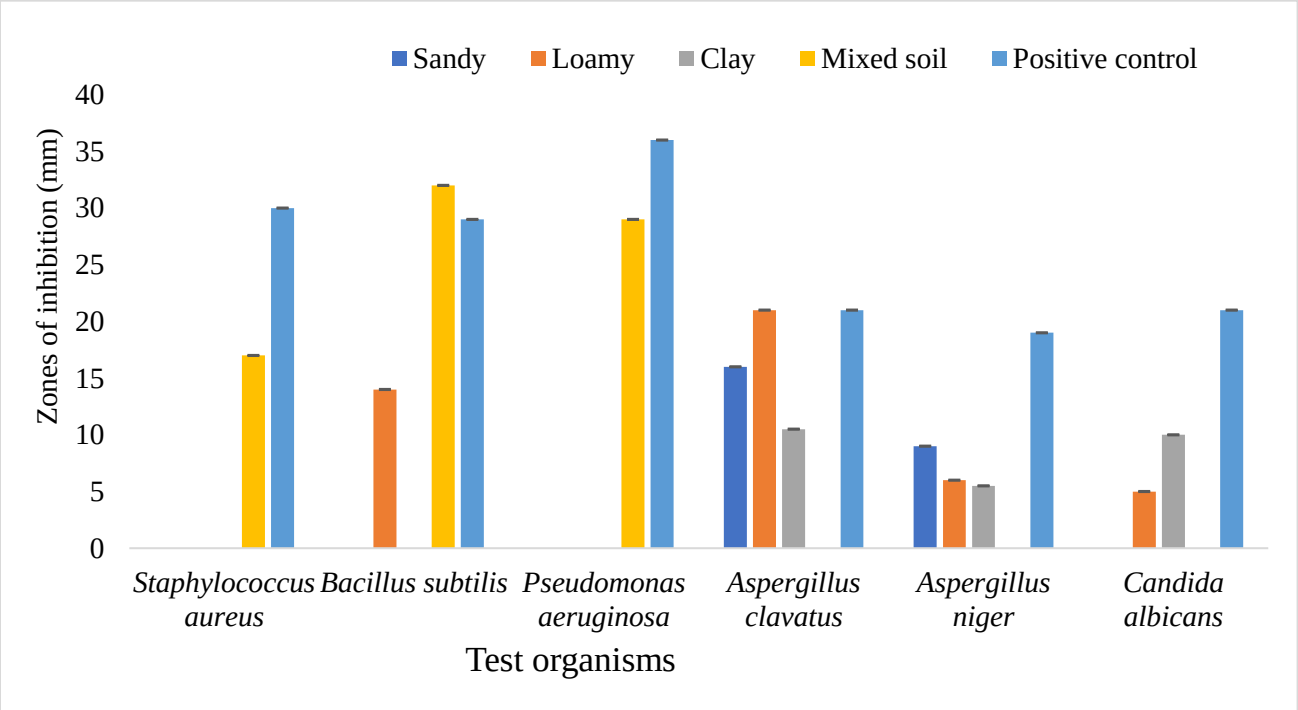

Figure 3: Zones of inhibition by the test organisms in the root extracts of *C. spectabilis* grown in different soil types.

Minimum Inhibitory Concentration (MIC) Assay

The MIC results on the clinical bacteria strains are represented in Table 4. The lowest MIC recorded was 0.625 µg/mL on *S. aureus* using extract of leaves grown in mixed soil, on *B. subtilis* from extract of leaves obtained from clay soil and extract of roots grown in mixed soil.

Table 4: Minimum inhibitory concentration of *Costus spectabilis* extracts against sampled organisms

Test Organisms	Minimum Inhibitory Concentration (µg/mL)							
	Sandy		Loamy		Clay		Mixed Soil	
	Leaves	Root	Leaves	Root	Leaves	Root	Leaves	Root
S. aureus	5	15	2.5	____	1.25	10	0.625	10
B. subtilis	2.5	10	2.5	5	0.635	___	1.25	0.625
P. aeruginosa	5	15	5	5	5	10	____	1.25
A. clavatus	15	5	5	5	____	10	5	___
A. niger	10	10	15	___	5	10	___	15
C. albicans	15	10	15	10	5	10	15	___

Proximate Contents of the Costus spectabilis from Different Soil Types

The results of the proximate contents analysis revealed that carbohydrates and proteins are the prominent contents in the leaf of *C. spectabilis*. It was observed that the highest percentage of carbohydrate (81.67 ± 0.02 %) and crude fiber (5.44 ± 0.05 %) were obtained from samples grown in loamy soil, while the highest percentage of protein (13.98 ± 0.01 %) was observed from samples grown in loamy soil. Crude fat was detected at a negligible percentage in all samples, the highest being 0.10 ± 0.01 % respectively samples obtained from sandy and silt soil. The moisture content was highest in the samples grown in silt soil at 1.22 ± 0.01 %. The proximate contents result is depicted in Table 5.

Table 5: Proximate contents of *Costus spectabilis* grown in different soil types

Soil Samples	Ash	Protein	Crude Fiber	Crude Fat	Moisture	Carbohydrate
%						
Clay	0.41±0.01	13.74±0.09	4.68±0.12	0.09±0.01	0.2±0.01	80.61± 0.03
Sandy	0.46±0.01	13.98±0.01	5.62±0.02	0.10±0.01	0.20±0.01	79.46±0.20
Loamy	0.40±0.01	12.03±0.02	5.44±0.05	0.09±0.01	0.24±0.00	81.67±0.02
Soil mixture	0.44±0.02	13.24±0.06	4.42±0.06	0.09±0.01	0.17±0.01	81.52±0.01
Silt	1.38±0.02	13.55±0.05	5.44±0.02	0.10± 0.01	1.22±0.01	80.30±0.02

*Values are expressed as mean ± standard error

Discussion

Costus spectabilis (yellow trumpet), is one of the symbols of Nigeria's coat of arms which depicts the national plant, emphasizing the country's reliance on natural resources. *C. spectabilis* has not been exploited for its medicinal potential. Thus, the preliminary phytochemical analysis, antimicrobial properties and proximate contents of its ethyl ethanoate leaf and root extracts were studied. The ethyl ethanoate extract yield of the leaves and roots harvested from plants grown in different soil types; sand, clay, loam, silt and mixed soil showed that the loamy soil extracts had the highest percentage yield. This could probably be because loamy soil is known to retain moisture and nutrients that can support plant growth and development. Van Averbeke et al. (2007)

submitted that soil with different texture have different physical conditions of different water holding capacity, drainage, nutrient composition, friability and aeration. The extract yield also showed that this plant can thrive in different soil types like other plants, such as ginger and banana, belonging to the same order- Zingiberales. Kandiannan et al. (1996) study on the growth of ginger in different soil types showed that the plant can do well in all soil types.

The preliminary phytochemical screening of the ethyl ethanoate leaf and root extracts of *C. spectabilis* revealed the presence of alkaloids, flavonoids, phenol, saponin, glycosides, anthraquinone and tannin in varying quantities. This is in consonant with the report of Keïta et al. (2019) whose phytochemical study of the leaves of *C. spectabilis* revealed the presence of alkaloids, sterol, flavonoids, coumarins and triterpenes and reducing compounds in the methanolic extracts. Although, only minute quantities were observed in most of the phytochemicals tested. This could be attributed to the choice of solvent used in the extraction process. Shehu et al. (2022) reported that a furostan saponin isolated from the rhizome of *C. spectabilis* exerts a cataract ameliorative effect *in vitro*.

In the antimicrobial assay, the leaf extracts of *C. spectabilis* grown in all soil types (clay, sandy, loamy and mixed soil) were potent against the bacterial strains *Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis* as significant inhibitory levels were noticed in caparison with the positive control experiments. This could probably be as a result of the synergetic effects of the phytochemicals observed in the plant samples. Phenolic compounds have been reported to inhibit microbial enzymes of bacterial cells at the same time increasing affinity of cytoplasmic membrane thereby inhibiting bacterial growth (Miklasińska-Majdanik et al., 2018). Miklasińska-Majdanik et al. (2018) also stated that the synergy of polyphenols like flavonoids and phenols can inhibit the growth of both Gram-positive and Gram-negative bacteria. This present study is in agreement with Bouarab-Chibane et al. (2019) who assessed the antibacterial activity of 36 phenolic compounds on six bacterial strains by monitoring the cell growth through the broth microdilution method. They reported that the Gram-positive strains were more sensitive to phenolic compounds than the Gram-negative strains. We observed that only the root extract of *C. spectabilis* grown in mixed soil showed inhibitory activities against all the bacterial strains tested. This also could be a synergetic effect of the phytochemicals. However, there was no significant effect of the leaf extracts of *C. spectabilis* on the fungal isolates assayed. It was noticed that the extracts only had fungicidal effects on *Aspergillus clavatus* with the exceptions of the leaf extracts from the plant grown in sandy and loamy soil, and root extract from plant grown in the mixed soil. The extracts only had fungistatic effects on *Aspergillus niger* and *Candida albicans* tested. This could be as a result of the little quantity of phytochemicals observed in the plant extracts which might not have been potent enough to inhibit the growth of the fungal species. Leontopoulos et al. (2015) reported in their study on in vivo and in vitro effect of different formation of polyphenolic compounds on the growth of some pathogenic fungi that the use of low concentration of liquid polyphenols at 5 and 10 % could control some cases of fungal pathogens, but higher concentration of 20 and 30 % can have phytotoxic effects on the pathogens. For instance, only a trace quantity of tannin was observed in all the extracts from the *C. spectabilis* leaves and roots. Tannin compounds are known to inhibit fungal growth at high concentrations (Nichols-Orians, 1991). Anthraquinones which are known effective antifungal compounds (Barros et al., 2011 and Friedman et al., 2020) were only detected in very minute quantity except in the extracts of leaf obtained from the plant grown in clay soil. This could explain the fungicidal effect on the *Aspergillus* species of the extracts of plant samples grown in clay soil.

The minimum inhibitory concentration which is the lowest concentration of antibacterial agent expressed in mg/L (µg/L) that prevents visible microorganism growth after overnight incubation (Kowalska-Krochmal and Dudek-Wicher, 2021) was observed to be lowest in the leaf extract of *C. spectabilis* grown in clay soil and root extract of plant sample grown in mixed soil against *B. subtilis* while it was lowest in the extract of leaves obtained from samples grown in the mixed soil against *S. aureus*. These strains could be susceptible to the extracts. However, further analyses need to be carried out to ascertain the

susceptibility as preliminary analysis and crude extracts were utilized in this present study. Kowalska-Krochmal and Dudek-Wicher (2021) noted that differences in the degree of a strain's susceptibility to antibiotics cannot be assessed by mere comparison of MIC values, which most likely can give an erroneous report. Standard guidelines from regulatory bodies such as CLSI (2018) and EUCAST (2021) are required before assertion.

The proximate contents of *C. spectabilis* leaves grown on different soil types showed that carbohydrates and proteins are the major nutrient contents. This could probably serve as a source of protein and carbohydrates for animal feed and human consumption. However, the toxicological analysis of the plant must be ascertained before use. This is in consonant with the findings of Anyasor et al. (2014) who reported a high carbohydrate content of 55.83 ± 3.71 % in the leaf of *Costus afer* which belong to the same family of Costaceae and Genus with *C. spectabilis*. It was noticed that *C. spectabilis* leaf samples have low moisture content in comparison with other species from the same genus such as *C. igneus* and *C. afer* as reported by Pazhanichamy et al. (2010) and Anyasor et al. (2014) to respectively contain 21.2 ± 0.447 % and 18.63±2.11 % moisture content.

Conclusion

The ethyl ethanoate extracts of the leaf and root of *C. spectabilis* harvested from different soil types showed the presence of phytochemical compounds which can be relevant for therapeutic purposes. The plant extracts also showed promising antimicrobial properties requiring further characterization. The proximate content revealed that the plant can be a reliable source of nutrients to supplement animal feeds and protein consumption for human. However, clarity on the toxicity of the plant is needed.

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Competing interests

The authors declare that they have no conflict of interest.

Author contribution

Abimbola Esther Bankole conceived and designed the project, finding the methodology and research planning, supervised of the research study, and was responsible for writing the original research paper.
Abosede Margaret Ebabhi was involved in writing part of the research paper, editing the methodology, and project administration
Caroline Umebese was responsible for finding a methodology, project administration, and the investigation process.
Folarin Owagboriaye contributed to the data gathering and application of statistical analysis.
Emmanuel Thomas was involved in the plant collection and sampling, the process of finding the research concept, and planning.

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