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Phytochemical Characterization and Antimicrobial Activities of *Costus afer* Ker Gawl. (Zingiberaceae) from Southwestern Nigeria for Medicinal Applications.

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Abstract: *Medicinal plants remain a vital source of bioactive compounds for drug discovery, particularly in developing countries where herbal medicine plays a key therapeutic role. Phytochemical composition refers to the variety and concentration of naturally occurring chemical compounds in plants, such as alkaloids, flavonoids, tannins, and saponins that are responsible for many of their biological and therapeutic effects. Antimicrobial activities, on the other hand, describe the ability of these compounds to inhibit or destroy the growth of pathogenic microorganisms, including bacteria and fungi. This study investigated the phytochemical composition and antimicrobial activities of Costus afer Ker Gawl. (Zingiberaceae) collected from Ede, Osun State, Nigeria. Fresh leaves and stems were air-dried, powdered, and extracted with 95% ethanol. Standard phytochemical assays were performed to quantify tannins, flavonoids, alkaloids, saponins, terpenoids, steroids, glycosides, and chlorophyll, while antimicrobial activity was assessed against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger, and Mucor spp. using the agar well diffusion method. Results revealed that both leaf and stem extracts contained significant levels of secondary metabolites, with the leaf extract showing higher concentrations of tannins (68.91 mg/100 g), flavonoids (22.61 mg/100 g), saponins (52.41 mg/100 g), and steroids (48.13 mg/100 g) compared to the stem. The leaf extract exhibited greater antibacterial activity, showing inhibition zones of 11.66 mm (S. aureus), 10.57 mm (E. coli), and 8.98 mm (P. aeruginosa), while both leaf and stem extracts demonstrated strong antifungal activity against C. albicans (22.57 mm and 22.36 mm, respectively). The results confirm that C. afer possesses potent antimicrobial properties, attributable to its rich polyphenolic and saponin content, thereby validating its traditional medicinal use. Comparative evaluation with Costus igneus indicates that C. afer exhibits higher phytochemical diversity and broader pharmacological potential. The study concludes that C. afer represents a promising natural source for developing plant-based antimicrobial agents and recommends further characterization using GC–MS, HPLC, and FTIR techniques, alongside toxicity and formulation studies to support its safe therapeutic application.*

Keywords*: Antimicrobial activity, Costus afer, Costus igneus, phytochemicals, secondary metabolites, traditional medicine*

1.0 Introduction

Medicinal plants remain an invaluable resource in the search for novel therapeutic agents, particularly in developing countries where reliance on herbal medicine is still widespread. Among these plants, *Costus afer* Ker Gawl. (commonly known as bush cane, ginger lily, or spiral ginger) has gained considerable scientific and ethnomedicinal attention due to its diverse biological activities. Belonging to the family Costaceae, *C. afer* is a perennial herb widely distributed across tropical Africa, including Nigeria, where it thrives in forest undergrowth and moist habitats

(Omokhua, 2011). Interestingly, *C. afer* is regarded as a close relative or sister species to *Costus igneus*, commonly known as the "insulin plant, (Fig 1.1)" which is globally acclaimed for its hypoglycemic effects and its traditional use in managing diabetes mellitus. This phylogenetic relationship suggests possible biochemical and pharmacological similarities that merit scientific exploration.

Fig 1.1. ***Costus igneus*** ***Costus afer***

Ethnomedicinally, *C. afer* is utilized in the management of numerous health conditions, including diabetes, arthritis, stomach ache, inflammation, and gout (Divengi *et al*., 2022; Thakur *et al*., 2016; Boison *et al*., 2019). In several parts of Nigeria, decoctions and extracts from its leaves, stems, and rhizomes are employed to treat cough, malaria, eye defects, measles, and other ailments, while also being used in traditional rituals for their perceived protective and aphrodisiac properties (Omokhua, 2011). The plant's therapeutic versatility is attributed to its rich content of secondary metabolites; flavonoids, alkaloids, saponins, tannins, phenols, terpenes, and glycosides, which contribute to its antioxidant, anti-inflammatory, and antimicrobial properties (Anyasor *et al*., 2010; Ezejiofor *et al*., 2013; Chioma *et al*., 2020).

Phytochemical and pharmacological investigations on *C. afer* have revealed significant antioxidant activity in its leaves and roots, with the leaves exhibiting high polyphenolic content and the roots demonstrating notable inhibitory effects comparable to vitamin C (Karime *et al*., 2020). Extracts from the stem and rhizome have also shown hepatoprotective effects, reducing oxidative stress induced by carbon tetrachloride in experimental rats (Chibueze *et al*., 2013). These findings support the therapeutic potential of *C. afer* and justify continued scientific evaluation of its bioactive constituents.

Despite numerous studies on *C. afer* across different regions of Nigeria, there remains a paucity of data on the phytochemical composition and antimicrobial potential of the species specifically growing in Ede, Osun State. Environmental factors such as soil type, altitude, and climatic conditions are known to influence the phytochemical profiles of medicinal plants, which can, in turn, affect their bioactivity. Consequently, a region-specific investigation of *C. afer* from Ede is essential to determine whether its phytochemical constituents and antimicrobial properties are consistent with or distinct from those reported elsewhere.

Furthermore, with the increasing global burden of multidrug-resistant microbial infections and the escalating cost of conventional antibiotics, there is a pressing need for alternative, affordable, and effective antimicrobial agents derived from natural sources. Plant-based antimicrobials such as *C. afer* could provide accessible therapeutic solutions, particularly in resource-limited settings.

Therefore, this study aims to identify and characterize the various phytochemical compounds present in *Costus afer* extracts obtained from Ede, Osun State, and to assess their antimicrobial effectiveness against selected bacterial and fungal strains. Specifically, the objectives are:
i. To conduct a proper identification of the plant in a standard laboratory.
ii. To perform phytochemical analysis on the extracts of the leaf and stem of *C. afer*.
iii. To assess the antibacterial properties of *C. afer* against selected bacterial strains.
iv. To evaluate the antifungal properties of *C. afer* against selected fungal strains.

2.0 Methodology

2.1 Materials

The materials and reagents used in this study included: conical flasks (250 mL), test tubes, Petri dishes, inoculating wire loop, spirit lamp, beakers, ethanol (95%), aluminum foil, test tube rack, nutrient agar, potato dextrose agar, volumetric flasks, measuring cylinders, cotton wool, and analytical weighing balance. Fresh leaves and stems of *Costus afer* were collected and used for extraction and phytochemical analyses.

2.2 Collection and Identification of Plant Species

2.2.1 Plant Collection

Fresh leaves and stems of *Costus afer* were harvested from naturally growing plants at Alesinloye market, Ibadan, Oyo State, Nigeria (latitude 7.3897051°N, longitude 3.87086°E). The samples were collected using sterile tools, placed in labeled polyethylene bags, and transported to the laboratory for further processing.

2.2.2 Plant Identification

The plant was taxonomically identified and authenticated at the Forestry Research Institute of Nigeria (FRIN), Jericho, Ibadan, Oyo State. A voucher specimen was deposited in the FRIN herbarium for reference.

2.3 Preparation and Extraction of Plant Materials

The leaves and stems of *C. afer* were air-dried at room temperature (25–28°C) for four weeks and monitored until constant weights were obtained. The samples were then oven-dried at 40°C to ensure complete dryness without burning and ground into fine powder using a mechanical grinder. For extraction, 100 g of the powdered sample was soaked in 500 mL of 95% ethanol in a conical flask, sealed with cotton wool and aluminum foil, and left to stand at room temperature for 72 hours with intermittent shaking. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure at 40°C to obtain the crude ethanolic extract, which was stored in sterile containers at 4°C until use (Jha and Sit, 2022).

2.4 Phytochemical Analysis

Preliminary phytochemical screening was conducted to determine the presence and quantities of major secondary metabolites using standard methods as cited below.

2.4.1 Determination of Total Flavonoid Content

The total flavonoid content was determined using the aluminum chloride colorimetric method described by Chang *et al*. (2002). Briefly, 50 µL of extract (1 mg/mL) was diluted with methanol to 1 mL, mixed with 4 mL distilled water, and 0.3 mL of 5% $NaNO_2$. After 5 minutes, 0.3 mL of 10% $AlCl_3$ was added, followed by 2 mL of 1 M NaOH after 6 minutes. The final volume was adjusted to 10 mL with distilled water. Absorbance was measured at 510 nm using a UV–VIS spectrophotometer, and total flavonoid content was calculated from a catechin standard curve and expressed as mg catechin equivalents (CAT/g dry weight). This process followed the method of Shraim *et al*. (2021).

2.4.2 Determination of Tannins

Tannin content was quantified following the AOAC method with modifications. One milliliter of extract was mixed with 20 mL distilled water, 2.5 mL Folin–Denis reagent, and 10 mL of 17% Na_2CO_3. The mixture was made up to 100 mL with distilled water, mixed thoroughly, and allowed to stand for 20 minutes. Absorbance was read at 760 nm, and tannin concentration was calculated from a tannic acid standard curve ($R^2 = 0.9818$) and expressed as mg tannic acid equivalents/100 mL.

2.4.3 Determination of Saponins

Saponin content was determined using the spectrophotometric method of Brunner (1984). One milliliter of extract was mixed with 100 mL isobutyl alcohol and agitated for 5 hours, then filtered. Twenty milliliters of 40% $MgCO_3$ was added to the filtrate and re-filtered. One milliliter of the resulting colorless solution was mixed with 2 mL of 5% $FeCl_3$ in a 50 mL volumetric flask, and the final volume was made up with distilled water. After 30 minutes, absorbance was read at 380 nm, and saponin content was calculated using standard saponin solutions (0–10 ppm).

2.4.4 Determination of Alkaloids

Alkaloid content was estimated using the alkaline precipitation gravimetric method (Harborne, 1973). A portion of the sample was macerated in 10% acetic acid in ethanol (1:10 w/v) for 4 hours, filtered, and concentrated to one-quarter of its original volume. Concentrated NH_4OH was added dropwise until alkaloid precipitated. The precipitate was filtered, washed with 1% ammonia, dried at 80°C, and weighed. The alkaloid content was expressed as a percentage of sample weight.

2.4.5 Determination of Chlorophyll Content

Chlorophyll was determined as described by Yoshida *et al*. (1971). One gram of the sample was extracted with 80% acetone, and the absorbance of the filtrate was measured at 645 nm and 663 nm. Chlorophyll a, b, and total chlorophyll were calculated as follows:

The total chlorophyll content =

$$Chl\ A = 12.7\ X\ ABS\ 663 - 2.69\ X\ ABS\ 645$$

$$Chl\ B = 22.9\ X\ ABS\ 645 - 4.68\ X\ ABS\ 663$$

$$Total\ chlorophyll = Sum\ of\ Chl\ A\ and\ Chl\ B$$

Results were expressed as mg/100 g of sample.

2.4.6 Determination of Steroids

Steroid content was determined according to Trease and Evans. Two milliliters of extract solution (prepared from 2.5 g of plant material in 50 mL distilled water) was mixed with 3 mL of 0.1 M NaOH (pH 9), followed by 2 mL chloroform and 3 mL ice-cold acetic anhydride. Two drops of concentrated H_2SO_4 were added carefully, and absorbance was measured at 420 nm. (Kaleta *et al*., 2024)

2.4.7 Determination of Terpenoids

Terpenoid content was determined by the method of Harborne (1973). About 0.8 g of powdered sample was extracted with 10 mL methanol and filtered. To 5 mL of filtrate, 2 mL chloroform and 3 mL concentrated H_2SO_4 were added. The reddish-brown coloration confirmed the presence of terpenoids. Absorbance was read at 538 nm for quantitative estimation.

2.4.8 Determination of Glycosides

Cardiac glycosides were quantified using the Baljet reagent method (El-Olemy *et al*., 1994). One gram of the sample was soaked in 10 mL of 70% ethanol for 2 hours and filtered. The filtrate was purified with lead acetate and Na_2HPO_4, followed by addition of freshly prepared Baljet reagent (95 mL aqueous picric acid + 5 mL 10% NaOH). Absorbance was read at 495 nm, and results were expressed as mg glycoside equivalents/g sample.

2.5 Test Microorganisms

Test organisms included Staphylococcus aureus (Gram-positive), Escherichia coli and Pseudomonas aeruginosa (Gram-negative bacteria), and fungal strains *Aspergillus niger, Mucor spp.*, and *Candida albicans*. Bacterial and fungal isolates were maintained on Nutrient Agar (NA) and Potato Dextrose Agar (PDA), respectively, at 4°C until use.

2.6 Preparation of Culture Media

Nutrient Agar and Potato Dextrose Agar (HiMedia, India) were prepared according to manufacturer's instructions. Media were sterilized at 121°C and 15 psi for 15 minutes, cooled to 45°C, and poured aseptically into sterile Petri dishes to solidify. Ethanol was used as solvent control.

2.7 Preparation of Inoculum

Stock cultures were maintained at 4°C. Active cultures were prepared by inoculating a loopful of each organism into nutrient broth (for bacteria) or PDA broth (for fungi). The broths were incubated at 37°C for 24 hours for bacteria and at 30°C for 7 days for fungi. The cell suspensions were adjusted to approximately 2.0×10^6 CFU/mL for antimicrobial testing.

2.8 Antimicrobial Assay (Agar Well Diffusion Method)

The antimicrobial activity of *C. afer* extracts was evaluated using the agar well diffusion method. Standardized microbial suspensions were spread evenly on the surface of solidified agar plates. Wells of 6 mm diameter were made aseptically using a sterile cork borer, and 0.1 mL of each extract was introduced into the wells. Ethanol served as the negative control, while Nystatin (300 µg/well) was used as the standard antifungal agent. Plates were incubated at 37°C for 24 hours for bacteria and at room temperature for 72 hours for fungi. The antimicrobial activity was expressed as the diameter (mm) of inhibition zones surrounding the wells.

3.0. Results and Discussion

3.1 Results

3.1. 1. Identification of Plant Result

The plant was identified as *C. afer* Ker Zingberaceae with reference number: FHI. 113918. An herb with lanceolate leaves, flowered pink.

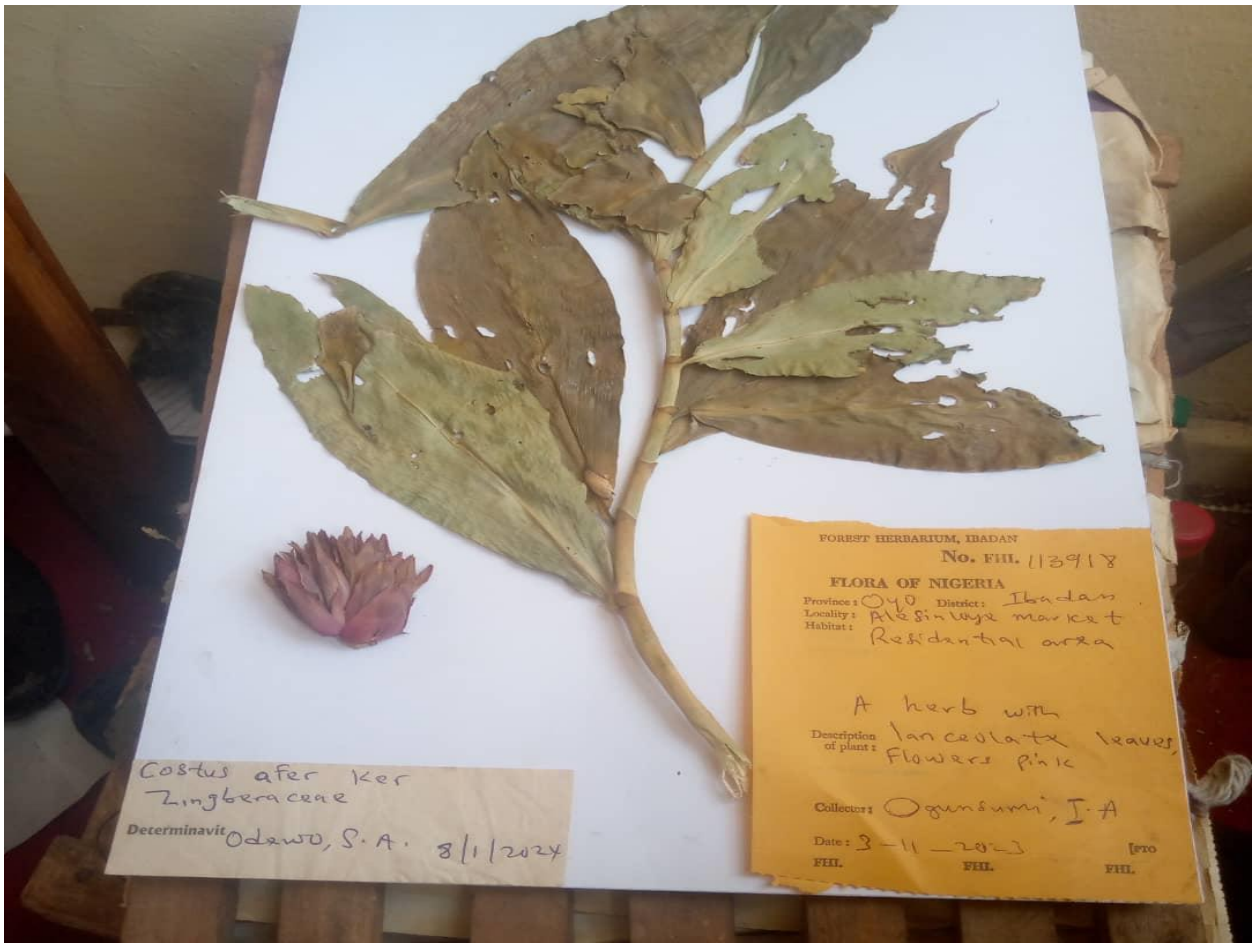

Plate 1: *Costus afer* Ker Zingiberaceae identified at FRIN, Ibadan

3.1.2 Phytochemical results of *C. igneus*

Table 3.2.1: The phytochemical composition of *Costus igneus* (Curled from Kumar, 2014)

Phytochemicals present in *Costus igneus*	Part of the plant	Solvent Extraction Material (Methanol)
Alkaloids	Leaf	Present
	Stem	Absent
Flavonoids	Leaf	Present
	Stem	present
Saponins	Leaf	Absent
	Stem	Absent
Glycosides	Leaf	Present
	Stem	Absent
Tannins	Leaf	Present
	Stem	Present
Terpenoids	Leaf	Absent
	Stem	Present
Anthraquinones	Leaf	Absent
	Stem	Absent
Quinones	Leaf	Present
	Stem	Present
Carbohydrates	Leaf	Present

	Stem	Absent
Steroids	Leaf	Absent
	Stem	Absent

Table 3.2.2 Phytochemical results of *C. afer* stem extract.

Sample name	Tannin	Flavonoids	Terpenoids	Saponins	Alkaloids	Steroids	Glycosides	Chlorophyll
***C. afer* Stem**	mg/100g	mg/100g	mg/100g	mg/100g	%	mg/100g	mg/100g	mg/100g
	46.54	9.06	14.76	22.12	5.86	20.19	8.46	17.50
	47.19	9.11	14.84	22.11	5.92	20.15	8.69	17.62
	46.83	9.02	14.86	22.16	5.87	20.17	8.58	17.58
Average	**46.85**	**9.06**	**14.82**	**22.13**	**5.88**	**20.17**	**8.58**	**17.57**

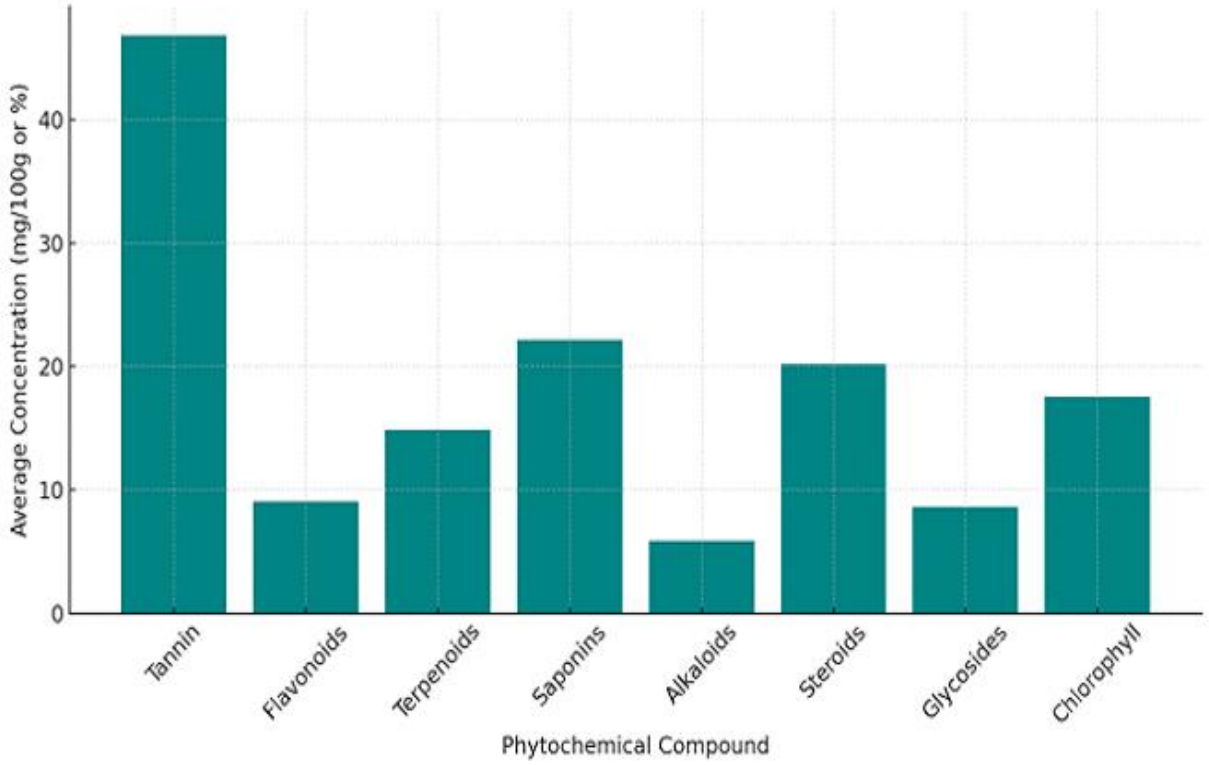

Fig 2.1: Average Phytochemical Composition of *Costus afer* Stem Extract

Table 3.2.3 Phytochemical results of *C. afer* leaf extract.

Sample name	Tannin	Flavonoids	Terpenoids	Saponins	Alkaloids	Steroids	Glycosides	Chlorophyll
***C. afer* Stem**	mg/100g	mg/100g	mg/100g	mg/100g	%	mg/100g	mg/100g	mg/100g
	68.47	22.56	28.67	52.32	8.65	48.09	19.32	47.62
	69.52	22.78	28.92	52.48	8.72	48.19	19.42	47.82
	68.75	22.49	28.53	52.42	8.94	48.12	19.16	47.62
Average	68.91	22.61	28.71	52.41	8.77	48.13	19.30	47.69

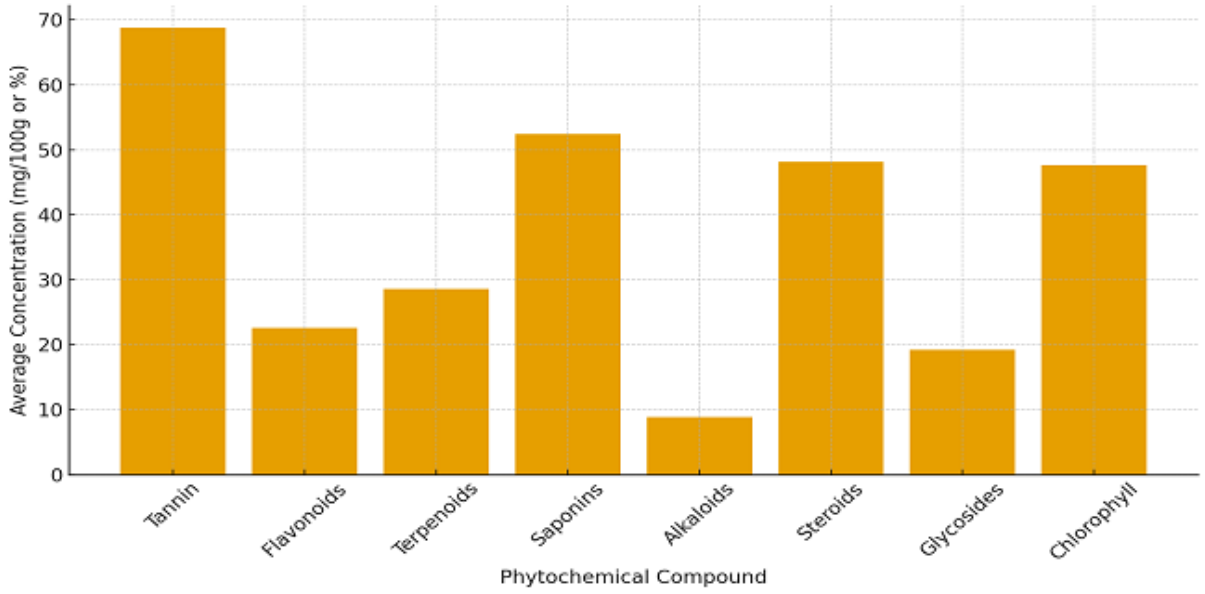

Fig 2.2: Average Phytochemical Composition of Costus afer Leaf Extract

3.1.3. Antimicrobial Susceptibility Results

The antibacterial and antifungal activities of the isolates; *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* was evaluated using the agar well diffusion method. The results, showing the inhibition zones (in millimeters) for Sample A (leaf extract) and Sample B (stem extract), are presented in Table 3.3.1 and 3.3.2

Table 3.3.1 Antibacterial Results of *C. afer* Leaf and Stem Extracts.

Sample ID	Solvent Used	EC	SA	PA
A (Leaf)	**Ethanol**	10.56	11.65	8.99
		10.58	11.65	8.96
		10.56	11.67	8.98
Average		**10.57**	**11.66**	**8.98**
B (Stem)	**Ethanol**	9.36	10.11	7.31
		9.37	10.11	7.33
		9.37	10.12	7.32
Average		**9.37**	**10.11**	**7.32**

Note: *E.C – Escherichia coli, S.A- Staphylococcus aureus, P.A- Pseudomonas aeruginosa*

Table 3.3.2 Antifungal Results of *C. afer* Leaf and Stem Extracts.

Sample ID	Solvent Used	CA	AN	MS
A (Leaf)	**Ethanol**	22.57	13.61	3.45
		22.58	13.69	3.45
		22.56	13.64	3.44
Average		**22.57**	**13.65**	**3.45**
B (Stem)	**Ethanol**	22.36	18.99	5.34
		22.37	18.98	5.34
		22.35	18.99	5.34
Average		**23.35**	**18.99**	**5.34**

Note: *CA- Candida albicans, AN- Aspergillus niger, MS- Mucor spp (SP)*

3.2 Discussion

4.2.1 Phytochemical Constituents of *Costus afer* (Stem and Leaf Extracts)

The qualitative and quantitative phytochemical screening of *Costus afer* revealed the presence of several bioactive compounds including tannins, flavonoids, terpenoids, saponins, alkaloids, steroids, glycosides, and chlorophyll in varying concentrations. The leaf extract consistently showed higher levels of these secondary metabolites compared to the stem extract. Specifically, tannin (68.91 mg/100 g), flavonoid (22.61 mg/100 g), saponin (52.41 mg/100 g), and steroid (48.13 mg/100 g) contents were markedly higher in the leaf extract than in the stem extract (46.85 mg/100 g, 9.06 mg/100 g, 22.13 mg/100 g, and 20.17 mg/100 g respectively). This observation agrees with the findings of Anyasor *et al*. (2014) and Sonibare *et al*. (2023), who reported that *C. afer* leaves generally possess higher concentrations of polyphenolic compounds than the stems or rhizomes, likely due to their greater photosynthetic activity and exposure to light, which promotes phenolic biosynthesis.

The abundance of these phytochemicals is pharmacologically significant. Tannins and flavonoids are well known for their antioxidant, antimicrobial, and anti-inflammatory activities (Chagas *et al*., 2022; Mora *et al*., 2022). Similarly, saponins and alkaloids contribute to membrane disruption in microbial cells and enhance immunomodulatory functions (Efimova and Ostroumova, 2021). The relatively lower concentration of alkaloids in both extracts (5.88% in stem and 8.77% in leaf) aligns with the findings of Chioma *et al*. (2020), who noted that *C. afer* exhibits moderate alkaloid content compared to other members of the Zingiberaceae family. The high chlorophyll and glycoside content observed in the leaf extract suggests the plant's potential for antioxidant and cardiotonic applications.

4.2 .2Antibacterial Activity of *Costus afer*

The antibacterial results demonstrated that both the leaf and stem extracts exhibited inhibitory effects against *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa* (with inhibition zones ranging between 7.32 mm and 11.66 mm. The leaf extract showed consistently higher activity than the stem extract across all tested organisms. The highest inhibition was observed against *S. aureus* (11.66 mm for leaf extract), followed by E. coli (10.57 mm) and *P. aeruginosa* (8.98 mm).

These results are consistent with the reports of Akpan *et al*. (2011) and Veiko *et al*. (2023), who also observed strong inhibitory activity of *C. afer* leaf extract against Gram-positive bacteria such as S. aureus, possibly due to the presence of tannins, flavonoids, and terpenoids that interfere with peptidoglycan synthesis. The lower susceptibility of P. aeruginosa aligns with previous findings by Odoemena and Ekanem (2019), who attributed this to the bacterium's thick outer membrane and efflux mechanisms that confer resistance to many natural compounds.

The higher antibacterial potency of the leaf extract over the stem extract may be linked to its greater concentration of phenolics and saponins, which can penetrate bacterial cell walls and induce protein coagulation and leakage of cellular constituents. This finding supports traditional claims that leaf preparations of *C. afer* are more effective in treating bacterial infections such as wounds, boils, and gastrointestinal disturbances.

4.2.3 Antifungal Activity of *Costus afer*

The antifungal assay revealed that both the leaf and stem extracts exhibited inhibitory activity against *Candida albicans, Aspergillus niger,* and *Mucor spp*. The leaf extract showed inhibition zones of 22.57 mm, 13.65 mm, and 3.45 mm, respectively, while the stem extract recorded 22.36 mm, 18.99 mm, and 5.34 mm for the same fungi. The highest antifungal activity was observed against *C. albicans*, while the least susceptibility was seen in *Mucor spp*.

This pattern is consistent with the findings of Coleman *et al*. (2010) and Yang *et al*. (2018) who reported that *C. afer* extracts possess strong antifungal activity, particularly against Candida species, due to the synergistic effects of saponins and terpenoids that disrupt fungal membrane integrity. The moderate activity against *A. niger and Mucor spp*. suggests that higher concentrations or prolonged exposure may be necessary to achieve fungicidal effects. Interestingly, the stem extract demonstrated slightly better inhibition of *A. niger* and *Mucor spp*. than the leaf extract, which may be due to the higher alkaloid-terpenoid interaction in stem tissues, supporting the report of Ituen and Udo. (2012) that stem phytochemicals sometimes exhibit synergistic antifungal properties.

When both species are compared, *C. afer* clearly demonstrates a richer and more diverse quantitative phytochemical composition than *C. igneus*, which exhibited a narrower qualitative spectrum. While *C. igneus* is noted for its specific bioactive properties, particularly those linked to diabetes management. *C. afer* possesses a broader range of secondary metabolites in higher concentrations, suggesting wider pharmacological potential. The abundance of tannins, saponins, and flavonoids in *C. afer* implies superior antioxidant and anti-inflammatory capabilities, while its significant chlorophyll content may enhance its detoxifying and wound-healing effects.

4.0 Conclusion and Recommendations

4.1 Conclusion

The findings from this study revealed that *Costus afer* (Zingiberaceae) contains significant quantities of bioactive phytochemicals that are responsible for its observed antimicrobial efficacy. The leaf extract exhibited a higher concentration of tannins, flavonoids, saponins, steroids, and glycosides compared to the stem extract, corresponding to stronger antibacterial and antifungal activities. Both extracts demonstrated moderate to high inhibitory effects against *S. aureus, E. coli, C. albicans, and A. niger*, validating the traditional medicinal use of *C. afer* in managing infectious diseases. The results confirm that the therapeutic potential of *C. afer* is largely due to its rich polyphenolic and saponin composition. Hence, the plant represents a valuable natural source for the development of antimicrobial agents that may serve as alternatives to synthetic drugs, especially amid rising antibiotic resistance.

The comparative phytochemical analysis reveals that both *Costus igneus* and *Costus afer* contain valuable bioactive compounds with therapeutic potential. However, *C. afer* exhibited higher concentrations and a broader diversity of phytochemicals in both leaf and stem extracts, indicating stronger pharmacological potential. *C. igneus*, though qualitatively rich in key compounds such as alkaloids and flavonoids, presents a more selective biochemical profile, supporting its specialized use in managing metabolic disorders. These findings highlight the complementary roles both species can play in herbal medicine, natural product formulation, and potential biotechnological applications.

4.2 Recommendations

Based on the findings of this study, further phytochemical characterization of *Costus afer* and *Costus igneus* is strongly recommended. Advanced analytical techniques such as Gas Chromatography-Mass Spectrometry (GC-MS), High-Performance Liquid Chromatography (HPLC), and Fourier Transform Infrared Spectroscopy (FTIR) should be employed to isolate, identify, and quantify the specific bioactive compounds responsible for the observed antimicrobial and therapeutic properties. This would provide a clearer understanding of the molecular constituents contributing to their pharmacological potentials.

In addition, comprehensive toxicological evaluation is essential to ascertain the safety profile of *C. afer* extracts for pharmaceutical and nutraceutical applications. In vivo and in vitro toxicity assessments should be conducted to determine safe dosage ranges, potential cytotoxic effects, and long-term safety implications. Establishing a clear toxicological baseline will facilitate the safe incorporation of *C. afer* into herbal formulations and drug development processes.

Formulation studies are also recommended to translate the therapeutic potential of *C. afer* into practical applications. The development of standardized herbal products such as ointments, capsules, gels, or syrups incorporating the plant extracts should be pursued to evaluate their stability, bioavailability, and efficacy under real-world conditions. This will bridge the gap between laboratory findings and clinical or industrial utilization.

Furthermore, synergistic studies combining *C. afer* extracts with conventional antibiotics should be explored to evaluate possible enhancement of antimicrobial activity against resistant bacterial strains. Such studies may provide insight into novel combination therapies capable of mitigating antimicrobial resistance, which is an increasing global health concern.

Given the higher quantitative presence of bioactive compounds in *C. afer*, detailed pharmacological and toxicological investigations are warranted to validate its therapeutic efficacy and potential for drug development. Comparative analyses between *C. afer* and *C. igneus* will help delineate their specific medicinal roles and identify possible synergistic interactions.

Finally, large-scale cultivation and conservation of both *C. afer* and *C. igneus* should be encouraged to prevent overharvesting from natural populations and to ensure sustainable raw material supply for industrial and medicinal applications. These species should also be integrated into sustainable agricultural systems not only for phytopharmaceutical use but also as potential bioresources in organic fertilizer production and waste recycling initiatives. Such an approach would align with environmental sustainability goals while promoting economic and health benefits through plant-based innovations.

References

Akpan, M. M., Odeomen, C. S., and Nwachukwu, C. N. (2011). Antimicrobial assessment of ethanolic extract of *Costus afer* leaves. *Asian Journal of Plant Science & Research*.

Anyasor, G. N., Onajobi, F. D., Osilesi, O., and Adebawo, O. (2014). Proximate composition, mineral content and in vitro antioxidant activity of leaf and stem of *Costus afer* (Ginger lily). *Journal of Intercultural Ethnopharmacology, 3(3), 128.*

Anyasor, G., Onajobi, F., Osilesi, O., Adebawo, O., and Oboutor, E. (2014). Anti-inflammatory and antioxidant activities of *Costus afer* Ker Gawl. hexane leaf fraction in arthritic rat models. *Journal of Ethnopharmacology, 155(1), 543–551.*

Boison, D., Adinortey, C. A., Babanyinah, G. K., Quasie, O., Agbeko, R., Wiabo-Asabil, G. K., and Adinortey, M. B. (2019). *Costus afer*: A systematic review of evidence-based data in support of its medicinal relevance. *Scientifica, 2019*.

Chagas, M. D. S. S., Behrens, M. D., Moragas-Tellis, C. J., Penedo, G. X., Silva, A. R., and Gonçalves-de-Albuquerque, C. F. (2022). Flavonols and flavones as potential anti-inflammatory, antioxidant, and antibacterial compounds. *Oxidative Medicine and Cellular Longevity, 2022(1), 9966750.*

Chioma, A. C., Albert, A. C., and Kenechukwu, A. J. (2020). Chemical profile of the stem extract of *Costus afer* (Bush Cane) from Imo State in Nigeria. *Asian Journal of Research in Biochemistry, 7(4), 113–123.*

Chibueze, F., Kingsley, N., Okorie, K., Akuagwu, A., and Success, N. (2013). Phytochemical composition of *Costus afer* extract and its alleviation of carbon tetrachloride–induced hepatic oxidative stress and toxicity. *Journal of Biological Sciences, 2, 120–126.*

Coleman, J. J., Okoli, I., Tegos, G. P., Holson, E. B., Wagner, F. F., Hamblin, M. R., and Mylonakis, E. (2010). Characterization of plant-derived saponin natural products against *Candida albicans*. *ACS Chemical Biology, 5(3), 321–332*.

Divengi, J., Idrissa, A., Kabasele, D., Mbuyi, P., Walle, I., Lesse, V., Kiala, R., Nzinga, P., Bongo, R., Ntezolo, J., Mukeba, F., Longo, B., Mutwale, P., Mesia, G., and Tona, G. (2022). *Costus afer* Ker Gawl (Costaceae): A review on its medicinal uses with focus on potential anti-inflammatory effect. *Journal of Pharmaceutical Research International.*

Efimova, S. S., and Ostroumova, O. S. (2021). Is the membrane lipid matrix a key target for action of pharmacologically active plant saponins? *International Journal of Molecular Sciences, 22(6), 3167.*

Ezejiofor, A., Orish, C. N., and Orisakwe, O. E. (2013). Effect of aqueous leaves extract of *Costus afer* Ker Gawl (Zingiberaceae) on the liver and kidney of male albino Wistar rat. *Ancient Science of Life, 33, 4–9.*

Ituen, E. B., and Udo, U. E. (2012). Phytochemical profile, adsorptive and inhibitive behaviour of *Costus afer* extracts on aluminium corrosion in hydrochloric acid. *Journal of Chemical Science and Technology.*

Jha, A. K., and Sit, N. (2022). Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. *Trends in Food Science & Technology, 119, 579–591*.

Kaleta, M., Oklestkova, J., Klicova, K., Kvasnica, M., Konickova, D., Mensikova, K., ... and Novak, O. (2024). Simultaneous determination of selected steroids with neuroactive effects in human serum by

ultrahigh-performance liquid chromatography–tandem mass spectrometry. *ACS Chemical Neuroscience, 15(10), 1990–2005.*

Karime, C., Marcelline, A., Anoubilé, B., Jean-Baptiste, K., Thibaut, B., Titah, J., Faustin, K., Daouda, B., and Claude, K. (2020). Phytochemical study and antioxidant activities on extracts of the leaves and roots of *Costus afer* Ker Gawl. (Zingiberaceae). *Oriental Journal of Chemistry, 36, 804–811.*

Kumar, N. P. (2014). *International Journal of Research in Advent Technology, 2(1).*

Mora, J., Pott, D. M., Osorio, S., and Vallarino, J. G. (2022). Regulation of plant tannin synthesis in crop species. *Frontiers in Genetics, 13, 870976.*

Odoemena, C. S., and Ekanem, N. G. (2006). Antimicrobial assessment of ethanolic extract of *Costus afer* leaves. *Journal of Science and Technology, 5(2), 51–54*.

Omokhua, G. E. (2011). Medicinal and socio-cultural importance of *Costus afer* (Ker Grawl) in Nigeria. *African Research Review, 5(5), 282–287.*

Shraim, A. M., Ahmed, T. A., Rahman, M. M., and Hijji, Y. M. (2021). Determination of total flavonoid content by aluminum chloride assay: *A critical evaluation. LWT, 150, 111932.*

Sonibare, M. A., Isola, A. O., and Akinmurele, O. J. (2023). Pharmacognostic standardisation of the leaves of *Costus afer* Ker Gawl. (Zingiberaceae) and Palisota hirsuta (Thunb.) K. Schum. (Commelinaceae). *Future Journal of Pharmaceutical Sciences, 9(1), 19.*

Thakur, M., Asrani, R., Thakur, S., Sharma, P., Patil, R., Lal, B., and Parkash, O. (2016). Observations on traditional usage of ethnomedicinal plants in humans and animals of Kangra and Chamba districts of Himachal Pradesh in North-Western Himalaya, India. *Journal of Ethnopharmacology, 191, 280–300.*

Veiko, A. G., Olchowik-Grabarek, E., Sekowski, S., Roszkowska, A., Lapshina, E. A., Dobrzynska, I., ... and Zavodnik, I. B. (2023). Antimicrobial activity of quercetin, naringenin and catechin: Flavonoids inhibit *Staphylococcus aureus*-induced hemolysis and modify membranes of bacteria and erythrocytes. *Molecules, 28(3), 1252.*

Yang, L., Liu, X., Zhuang, X., Feng, X., Zhong, L., and Ma, T. (2018). Antifungal effects of saponin extract from rhizomes of *Dioscorea panthaica* Prain et Burk against *Candida albicans*. *Evidence-Based Complementary and Alternative Medicine, 2018(1), 6095307.*