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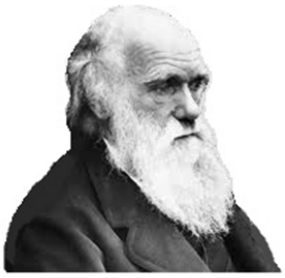

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Isolation of Flavonoids, Antioxidant and Antimicrobial Activities of *Costus afer* Ker Gawl. Stem Extracts

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ABSTRACT

Resistance to antimicrobial agents has become an increasingly important and pressing global problem. Hence, the need for substantial investment and research in the field of anti-infectives are now desperately needed if a public health crisis is to be averted. This study aims to determine the antioxidant and antimicrobial activity of *Costus afer* stem and isolate the flavonoids in the extracts. The method used for isolation was a combination of thin layer chromatography (TLC) and column chromatography (CC). Phytochemical screening tests were used for identification of the eluate fractions of CC to ascertain the flavonoid-rich fraction. The flavonoid content in dry stem extracts (DSE); 153 µg/g was lesser than that in fresh stem extracts (FSE) 186 µg/g. All the extracts showed activity against test organisms (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* spp.) in a concentration-dependent manner. There was an observed resistance of *S. aureus* against FSE at 25 mg/ml. *E.coli* and *Pseudomonas* spp were sensitive to DSE at almost all concentrations *Pseudomonas* spp was sensitive to almost all the control drugs except cefuroxime where it recorded a resistance. DPPH radical scavenging activity was positively correlated to the concentration of the stem extracts. FSE sample showed higher DPPH radical scavenging activity than DSE as evident in Table 3 and Fig. 2. The reducing power of the extracts followed the order of DSE < FSE < VITC. ABTS radical scavenging activity was also positively correlated to the concentrations of the stem extracts. In this analysis, FSE sample showed higher radical scavenging activity of ABTS than the DSE sample.

***Keywords*:** Flavonoids, Stem extract, DDPH, Antimicrobial, Antioxidant, *Costus afer*

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1. INTRODUCTION

Herbal remedies from medicinal plants are becoming increasingly popular as people seek natural treatments for health issues. Medicinal plants treat a variety of illnesses and conditions. They are known for their therapeutic properties which are of paramount importance in the medical and sociocultural fields (Nazaj, 2017). Additionally, they can be easily grown, harvested and prepared for use in a variety of ways. Plants contain active molecules called phytochemicals otherwise referred to as secondary metabolites which are accountable for its medicinal nature. Examples of such phytochemicals are flavonoids, tannins, alkaloids etc. (Bassey and Obi, 2018). Non-pharmaceutical compounds such as ginseng from green tea and compounds like penicillin are natural products used by humans for health benefits. Traditional herbal medicine continues to play an important role in the healthcare system, with around 60% of the world's population relying primarily on traditional medicines for health care. Modern knowledge of medicinal plant research still includes at least 25% of medicinal products and many others, which are synthetic analogues based on prototype compounds isolated from medicinal plants. Research has shown that phytochemicals have been employed in the studies of anti-inflammatory, anti-carcinogenic, anti-tumor, antioxidant activities etc. (Dutta, *et al*. 2014).

Costus afer, also known as African ginger or spiral ginger, is a tropical plant native to West and Central Africa. Commonly called bush reed or ginger lily, *C. afer* is known in Nigeria as “Kakizawa” in Hausa, “Mbritem” in Efik, “irekeomode” in Yoruba and “monkey sugarcane” by the Anglophone region of the Cameroon republic (Anyasor, *et al*. 2013). It belongs to the family *Costaceae* and has been traditionally used in African herbal medicine for its various medicinal properties. In Nigeria, the plant is used as part of a ritual to encourage childbirth in women. It is believed that consuming a decoction made from the leaves of the plant can help a pregnant woman deliver her child without complications. Several studies have investigated the phytochemical composition of *Costus afer*, which revealed the presence of bioactive compounds such as alkaloids, flavonoids, phenolic compounds, terpenoids, saponins, tannins, and glycosides (Bassey and Obi, 2012). These phytochemicals contribute to the plant's medicinal properties and may be responsible for its therapeutic effects.

The plant extracts have been effective against both gram-positive and gram-negative bacteria (Izunwanne, *et al*., 2016). Flavonoids are bioactive natural product molecules possessing polyphenolic structure, which are often found in fruit, vegetables and some beverages (Panche, *et al.* 2016). Polyphenols are chemicals in the secondary metabolism of plants that can accumulate in certain groups of plant organs such as leaves, fruits, roots and stems (Karak, 2018). The presence of 15 carbon atoms in the basic structure is a characteristic of flavonoids, arranged in the form C6-C3-C6, which corresponds to two aromatic rings A and B linked by a three-carbon atom unit ring. The rings are marked with the letters A, B and C (Dias, *et al*. 2021). Fig. 1 shows the structure of the basic skeleton of flavonoids.

They have attracted much interest over the last decade due to their multiple effects on human and animal health and their dominance in the plant kingdom. In literatures, they are been referred to as functional ingredients and health-promoting biomolecules due to their potential role in health promotion and prevention of chronic degenerative diseases. (Nijveldt, *et al.*, 2001). There are thousands flavonoids that contribute to the colourful pigments of fruits, herbs, vegetables and medicinal plants. They are also responsible for red and dark blue colour of berries, as well as orange and yellow colouring of citrus fruits (Matik and Gasik, 2009).

Kumar and Pandey, in 2013 conducted a research on the chemistry and biological activities of flavonoids and concluded that they influence the quality and stability of foods by acting as antioxidants. In a study conducted by Gutierrez-Venegas *et al*, (2019) on effect of flavonoids on antimicrobial activity of microorganisms present in dental plaque, some flavonoids (Quercertin and Morin) were tested against oral bacteria (*Actinomyces naeslundii* and *Actinomyces viscosus*)

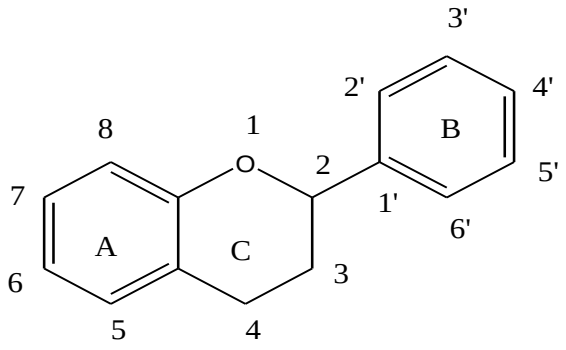

Figure 1. The basic skeleton structure of Flavonoid

Quercetin and morin showed antibacterial activity against *A. naeslundii* and *A. viscosus.* Quercetin showed an activity at 10 mg/ml (3.3×10^{-2} M) for *A. naeslundii* and 30 mg/ml (9.9×10^{-2} M) for *A. viscosus* while Morin at 10 mg/ml (3.3×10^{-2} M) for both strains.

However, it's important to note that the antimicrobial efficacy of specific flavonoids can vary depending on factors such as their chemical structure, concentration, and the target microorganism (Li *et al*., 2020).

2. RESULTS / EXPERIMENTAL

Materials

Beakers, Test tubes, Test tube rack/holder, Evaporating dish, Pipette, Weighing balance (Model number: AE436467), Rotary evaporator, Methanol(MeOH), Hydrochloric acid, (1%), Distilled water, Ferric chloride, Benedict reagent, Sodium hydroxide (2%), Ammonia solution, Benzene, Chloroform, Ascorbic acid (Vitamin C), Distilled Water.

Collection of plant materials and preparation of extracts

Fresh stems of *Costus afer* plant were harvested at Iterigbi community, Uvwie Local Government Area, Delta State. Authentication was done by the department of botany, Delta state university, Abraka. A portion was left to dry for 5 days. Both the fresh portion and dried portion where pulverized separately. The weight of the fresh and dried plant materials were 600g each. The extraction was carried out with methanol solvent (analytical grade) using soxhlet extractor. The samples were poured into the thimble chamber and the condenser was connected with inlet and outlet source for cooling the system during the extraction. The soxhlet set was connected to a round bottom with some volume (500 ml) of methanol in it. Then the round bottom flask was placed on a heating mantle connected to an electrical source and its

vapours condensed into the condenser. The condensed vapour dripped into the thimble containing the sample, and extracts it by contact. When the level of solvent in thimble rose to the top of siphon tube, the liquid contents of thimble siphoned into round bottom flask. The process was continuous. Each extract was concentrated with rotary evaporator to get oily samples and stored in glass containers. The weights of the oily samples were 25.7g for fresh stem extracts (FSE) and 21.4g for dry stem extracts (DSE). The colour of the dry sample extract was light greenish while that of the fresh sample was dark greenish.

Phytochemical screening

The phytochemical screening was conducted according to Musa, (2015) and Karime *et al*., (2020), with slight modifications. All extracts obtained were screened for their photochemical constituent using standard procedures.

a. Test for tannins

$FeCl_2$ (few drops) was added to the extracts (1 ml). A green precipitate indicated the presence of Tannins.

b. Test for Saponins (Frothings)

1 ml of each methanol extract was boiled with 5cm^3 of distilled water in a test-tube and filtered. Then 2.5 cm^3 of distilled water was added and shaken vigorously for a stable persistent froth. Thereafter, the frothing was mixed with some drops of olive oil and shaken vigorously. The formation of the emulsion indicated the presence saponins.

c. Test for flavonoids (Shinoda's test)

5 drops of HCl and 10 mg of Zn shavings were added to 2 ml of the extracts. After 3 minutes, a red-orange colour indicates the presence of flavonoids.

d. Test for alkaloids (Wagner's test)

1 ml of each extract was dissolved in 5 ml of 1% HCl on a steam bath. The solution obtained was filtered and 2 ml of the filtrate was treated with few drops of Wagner's reagent (Iodine in potassium iodide). Formation of brown or reddish-brown precipitate was regarded as evidence for the presence of alkaloids in the extracts.

e. Test for Glycosides

1 ml of the extract was treated with 2 ml of glacial acetic acid and one drop of ferric chloride solution added. This was under-laid with 1 ml of concentrated sulfuric acid. A brown ring of the interface indicates the presence of dioxygen sugar, characteristic of cardenolides.

f. Test for steroids

2 ml of acetic anhydride was added to 1ml of the extract and 2 ml of H_2SO_4 was added. A colour change from violet to blue or green indicates the presence of steroids.

g. Test for terpenes

1ml of the extract was mixed with 2 ml of chloroform and 3 ml of concentrated H_2SO_4 was carefully added to form a layer. The red-brown colour of the interface indicates the presence of terpenes.

Antimicrobial Analyses

Antimicrbial analysis was done according to Ogwuche and Amupitan, (2015) with modifications. The antimicrobial assay of the methanol extracts of *C. afer* were conducted using three microbes of medical importance such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas spp*. The microbes were obtained from the department of microbiology, University of Benin. Standard microbiological and biochemical procedures were followed in identifying each microbial strain. The concentrations of 25, 50, 75 and 100 mg/ml were made from the original extracts. 24-hour culture of bacteria suspension (*E. coli, Staphylococcus aureus* and *Pseudomonas* spp) were prepared, sterilized using peptone water. 0.5 ml Macfarland standard solution was used to standardize the culture. Mulleen-Hinton Agar were weighed, dissolved and later poured on petri dishes and allowed to solidify. 24-hours cultures of bacteria suspension were streaked on Mulleen-Hinton agar using a sterile wire or inoculating loop. Thereafter, sterile cork-borer was used to bore holes on the already streaked agar plate and they were well labelled accordingly to the concentrations of the extracts prepared. This was followed by introducing the extract to their respective holes bored on the agar plates. All the plates were incubated at a temperature of 37 °C for 24 hours. Upon incubation, a measuring ruler was used to measure the zone of inhibition in millimeter. The lowest zone of inhibition (value) in mm represents the minimum inhibition concentration (MIC).

Antibiotic Sensitivity Test

37.5g of Mulleen Hinton Agar was weighed dissolved into sterile 1000 ml of distilled water and autoclaved at 121 °C for 15 minutes. It was poured on sterile dishes and allowed to solidify. Bacterial isolates (*E. coli, Staphylococcus aureus*, and *Pseudomonas* spp) were streaked on the Mulleen Agar plates. All the plates were incubated at a temperature of 37 °C for 24 hours. Upon incubation, all the zones of inhibition were measured using a measuring ruler and it was calculated and recorded.

Antioxidant Analyses

DDPH Analysis

The analysis was carried out according to the method of Baliyan, *et al*., (2022) with modifications. The DPPH reagent had a solution concentration of 80 µg/ml and was prepared by dissolving 8 mg of DPPH in 100 ml of MeOH. 100 µl of DPPH reagents were combined with 100 µl of sample in a 96-well microplate, and the mixture was incubated at room temperature for 30 minutes to measure the scavenging activity. Ascorbic acid (Vitamin C) was employed as a control after incubation, and absorbance at 514 nm was measured with a microplate reader. The three sets of experiments were completed, and mean values were computed. Using the formula below, the DPPH scavenging effect was calculated:
Scavenged DPPH (%) = (AB-AA)/AB) 100

FRAP Analysis

According to prior instructions, FRAP solutions were prepared (Balaji *et al.* 2015), with modifications. 10 ml of TPTZ (2,4,6-Tripyridyl-S-Triazine) and 10 ml of ferric chloride were added to 10 ml of acetate buffer (30 mM, pH 3.6) to produce the FRAP solution. According to earlier reports (Jimenez-Alvarez *et al.,* 2008), the microplate FRAP assay was carried out. The

96-well microplate was initially filled with sample solutions (20 µl), and then with FRAP solution (280 µl). The mixtures were shaken, and incubated at 37 °C in the dark for 30 minutes, and then 593 nm readings were recorded using a microplate reader. The experiments were done in replicas of three and the mean values were calculated.

ABTS Analysis

In double-distilled water, ABTS 7mM and 2.45 mM potassium persulfate were added and dissolved. The solution was then diluted with distilled water in a 1:9 v/v ratio. 10 l of the sample/standard (ascorbic acid) was pipetted into a microtiter well after 190 l of reagent had been added. At 735 nm, absorbance was measured. A reagent blank reading was taken (A-control) and after the addition of the sample, the absorbance (A-sample) reading was taken 6 min after initial mixing. For calculating the antioxidant activity, values before the start of the decrease of the absorbance (A0–A6) and the last measurement value were used. The experiments were done in replicas of three and the mean values recorded. Values were deduced according to the formula:

$$\% \text{ Radical scavenged} = [A_{control} - A_{sample}/A_{control}] \times 100$$

$A_{control}$ = Absorbance of blank reagent
A_{sample} = Absorbance of sample after 6 minutes

Quantification of Flavonoid

Flavonoids content was determined according to Herald *et al.*, (2012) with some modifications. 1 ml of the extract was added to 0.5 ml of 5% Sodium Nitrite ($NaNO_2$) in a 10 ml volumetric flask. After 5 min, 0.5 ml of 10% $AlCl_3$ was pipetted into the volumetric flask and reaction was allowed to take place for 1 min. Also, 2 ml of 1 M NaOH was pipetted into the flask and the volume was made up to 10 ml with distilled water. The content of the flask was further mixed, kept for 30 minutes and the absorbance was measured at 420 nm using UV Spectrophotometer in triplicates. Various concentrations (10–150 µg/ml) were prepared from the stock solution of quercetin standard (1mg/ml) using serial dilution to obtain the standard calibration curve. The quantity of flavonoids in each sample was calculated as micrograms of quercetin equivalent per gram of extract, and a quercetin standard calibration curve was developed. Using the equation below, the flavonoid content was determined.

$$F = (C \times V) / M$$

where:

F = Flavonoid content in µg/g quercetin equivalent;
C = the concentration of quercetin established from calibration curve (µg/ml),
V = Extract Volume (ml),
M = Weight of extract (g)

Quantification of Phenol

Phenolic content was determined using the method of Zhang, *et al.*, (2006) with modifications. The crude extract was diluted with distilled water. To 2 ml of diluted extract, 1

ml of 10% FC reagent was added, and after 7 min, 1 ml of 7.5% sodium carbonate was added to the mixture.

The solution was mixed thoroughly and left to incubate for about 40 min. Afterwards, solution absorbance was measured at 765 nm using UV Spectrophotometer, in triplicates. A standard solution of gallic acid (10–150 µg/ml) was also prepared using serial dilution and the calibration curve was constructed. The concentration of phenols was expressed as µg GAE/g of extract. The phenolic content was calculated using the equation:

$$P = (C \times V) / M$$

where:

P = Gallic acid equivalent (GAE)/g of phenolic compounds
C = the gallic acid concentration determined by the calibration curve (g/ml).
V = Extract volume (ml), and
M = Weight of extract (g)

Isolation of Flavonoid

Isolation of flavonoid was performed according to Liu *et al.* (2018) with some modifications. A combination of thin-layer chromatography (TLC) and column chromatography (CC) methods were used in the isolation as well as identification. Firstly, TLC analysis was performed on the extract samples (DSE and FSE) recovered from the soxhlet extraction of the *C. afer* stem. On the TLC plate, a spot of the sample solution was placed 1 cm from the plate's base.

The TLC plate was added to a development tank along with an initial mobile phase (methanol), and the tank was then sealed. As the plate developed, sample components were separated and appeared as spots. To find the optimal fit for clean separations, a variety of solvent mixtures with various polarity and ratios were created and utilized as mobile phases. Chloroform/methanol with a solvent ratio of 70:30 gave clear separation spots.

The plate was taken out of the development tank once the solvent had almost reached the top of it, dried, and viewed using iodine crystal vapours. The Rf values were calculated for each spot.

The component separation was then carried out by column chromatography. The stationary phase used is silica gel (60-120 mesh), while the mobile phase used is chloroform: methanol (70:30).

Glass wool was first inserted into the column to pack it, and then silica gel was slurred.

The silica gel in the column was compacted before use so that the obtained separation was good. The extract, dissolved in a small quantity of methanol was then introduced through the top of the column and allowed to elute.

The resulting eluate was collected in fractions (5) and labeled in test tubes (A to E). The fractions in the test tubes were monitored by TLC and were found to have a range of Rf values. Each of the fractions was further identified by standard screening tests both for FSE and DSE.

The fractions were concentrated to remove the solvent. Antimicrobial and antioxidant analyses were performed on the fractions which tested positive to Shinoda's test for the presence of flavonoid compounds to ascertain their level of activity in comparison to that of the crude extracts.

3. DISCUSSION

Phytochemical Analysis

The actual and percentage yields are shown in Table 1. The preliminary phytochemical screening of DSE and FSE of *C. afer* stem was done to assess the presence of bioactive compounds and the results were presented in Table 2. The findings revealed that the majority of the phytochemical substances investigated were present in stem extracts. Phlobatanins and saponins weren't found in DSE, but anthraquinones weren't found in FSE either. According to Daniel *et al*., (2020), the numerous phytochemical substances found in stem extracts have a variety of physiological activity, therapeutic value, and health advantages.

Table 1. Yields of Dry (DSE) and Fresh (FSE)

Properties	DSE	FSE
Mass of sample (g)	600	600
Actual Yield (g)	21.4	25.7
Percentage Yield (%)	3.6%	4.3%
Colour	Light- green	Dark-green

Table 2. Phytochemical Screening of DSE and FSE

COMPOUNDS	INFERENCE	
	DSE	FSE
Alkaloids	+	+
Tannins	+	+
Saponins	—	+
Phlobatanins	—	—
Flavonoids	+	+
Anthroquinones	+	—
Terpenes	+	+
Steroids	+	+
Phenols	+	+
+ = Present ; — = Absent		

Isolation of Flavonoid

The results of the TLC analysis carried out on the crude FSE sample showed four distinct spots with R_f values of 0.06, 0.13, 0.24 and 0.60 respectively. The DSE sample gave Rf values of 0.32, 0.43 and 0.62. For eluates from FSE crude extracts, fraction A tested more positively to saponins screening and negative/slightly positive to other screening tests with Rf values of 0.15 to 0.17. This corresponds to values gotten for saponins in a study by Kim *et al*. (2015). Fractions B and C with Rf values of 0.24 and 0.33 respectively, tested positively to Wagner's screening for alkaloids and negatively to other screening tests.

This is in close agreement with values of alkaloids in a study by Johnson *et al*., (2020) for colchicine (0.20) and strychnine (0.32). And lastly, fractions D and E, reacted positively to Shinoda's test on the availability of flavonoids, giving a red-to-orange colour and also Rf values of 0.51 and 0.63 which are closer to values obtained for flavonoid compounds (Isoquercetin-0.52 and Chlorogenic acid-0.65) in a study by Johnson *et al*., (2020). For fractions from crude DSE, Fractions A and B tested positively to Wagner's alkaloid test and negatively to other tests. Fraction C tested positive to Shinoda's test for flavonoids with an Rf value of 0.72. This is close to the value of kaempferol (0.75) stated by Johnson *et al*., (2020). Fractions D and E reacted more positively to the ferric chloride test for tannins, also having Rf values of 0.43 and 0.47 respectively. These are in close agreement with the values of tannin (0.41), in research by Tano, *et al*., (2018).

Antimicrobial Activity

Tables 3 and 4, shows the result of the antimicrobial activity of dry and fresh stem extracts of *C. afer* respectively tested against three bacterial strains at different concentrations (25, 50, 75, and 100 mg/ml). The extent of sensitivity of the test organisms to the plant extracts was assessed by measuring the zone of inhibition after 24 hours of incubation. Ofloxacin drug was used as standard with a potency of 5 µg.

All the extracts showed activity against test organisms in a concentration-dependent manner. Results from Table 3 showed that *E.coli* and *Pseudomonas* spp were susceptible to DSE of *C. afer* at almost all concentrations. *Staphylococcus aureus* (*S. aureus*) showed noticeable growth inhibition at higher concentrations of 75 and 100 mg/ml. The minimum inhibitory concentrations for DSE against *E. coli* (11.0 mm), *S. aureus* (8.0 mm) and *Pseudomonas* (6.5 mm) were 25, 75 and 50 mg/ml respectively. The FDSE against *E. coli* was able to inhibit at three concentrations in contrast to *S. aureus* and *Pseudomonas* spp. which inhibited only at two concentrations. However, activity was minimal for FSE against *E. coli*, only showing inhibitions (6.0 mm and 10.0 mm) at concentrations of 75 and 100 mg/ml respectively.

The flavonoid fraction (FFSE) against *E. coli*, displayed observed inhibition only at 100 mg/ml unlike for *S. aureus* and *Pseudomonas* spp. where it was able to show inhibitions at two concentrations of 75 and 100 g/ml respectively. *S. aureus* was found to be resistant to FSE at a concentration of 25 mg/ml. The phytochemical compounds detected in the extracts may account for their level of antimicrobial activity (Edema *et al*., 2012).

The absence or extremely low concentration of additional phytochemicals such as alkaloids in the fractions may be the cause of the observed lower level of activity of FDSE and FFSE compared to DSE and FSE, respectively. Alkaloids are greatly responsible for the level of antimicrobial activities in plant extracts (Daniel and Etukudo, 2019).

Table 3. Antimicrobial Activities of DSE, FDSE and Standard

TEST ORGANISMS	CONCENTRATIONS (mg/ml)			
	25	50	75	100
E. coli (S)	15.0	19.0	26.0	32.0 (mm)
E. coli (DSE)	11.0	17.0	21.0	28.0 (mm)
E. coli (FDSE)	N/A	7.5	12.0	15.5 (mm)
S. aureus (S)	5.0	7.0	12.0	16.0 (mm)
S. aureus (DSE)	N/A	N/A	8.0	12.0 (mm)
S. aureus (FDSE)	N/A	N/A	N/A	7.5 (mm)
Pseudomonas (S)	6.0	8.0	13.0	15.0 (mm)
Pseudomonas (DSE)	N/A	6.5	10.0	13.0 (mm)
Pseudomonas (FDSE)	N/A	N/A	5.0	8.5 (mm)
N/A = No Activity; S = Ofloxacin (Standard)				

Table 4 shows the result of the activities of FSE and FFSE against the bacteria strains. The flavonoid fraction (FFSE) against *E. coli*, displayed observed inhibition only at 100 mg/ml unlike for *S. aureus* and *Pseudomonas* spp. where it was able to show inhibitions at two concentrations of 75 and 100 g/ml respectively. *S. aureus* was found to be resistant to FSE at a concentration of 25 mg/ml. The phytochemical compounds detected in the extracts may account for their level of antimicrobial activity (Edema et al., 2012). The absence or extremely low concentration of additional phytochemicals such as alkaloids in the fractions may be the cause of the observed lower level of activity of FDSE and FFSE compared to DSE and FSE, respectively. Alkaloids are greatly responsible for the level of antimicrobial activities in plant extracts (Daniel and Etukudo, 2019).

Table 4. Antimicrobial Activities of FSE, FFSE and Standard

TEST ORGANISMS	**CONCENTRATIONS (mg/ml)**			
	25	50	75	100
E. coli (S)	15.0	19.0	26.0	32.0 (mm)
E. coli (FSE)	N/A	N/A	6.0	10.0 (mm)
E. coli (FFSE)	N/A	N/A	N/A	5.2 (mm)

S. aureus	(S)	5.0	7.0	12.0	16.0 (mm)
S. aureus	(FSE)	N/A	5.0	7.0	12.0 (mm)
S. aureus	(FFSE)	N/A	N/A	5.5	8.5 (mm)
Pseudomonas	(S)	6.0	8.0	13.0	15.0 (mm)
Pseudomonas	(FSE)	N/A	6.0	9.0	12.5 (mm)
Pseudomonas	(FFSE)	N/A	N/A	6.5	10.0 (mm)
N/A = No Activity; S = Ofloxacin (Standard)					

Antioxidant Activity

From the results of three methods of antioxidant analyses carried out, it was observed that the degree of DPPH radical scavenging activity was positively correlated to the concentration of the stem extracts. Figure 2 reveal that the DPPH radical scavenging ability of the FSE sample was higher than that of the DSE sample. At the lowest concentration of 10 µg/ml, DSE showed a higher percentage activity of 14.25% than FSE (12.97%), but as the concentrations increased, the activity of FSE became higher than DSE when compared with the activity of the standard (Vitamin C). At the highest concentration of 150 µg/ml, the percentage activities of FSE and Vitamin C (VIT C) were 73.83% and 80.77% respectively.

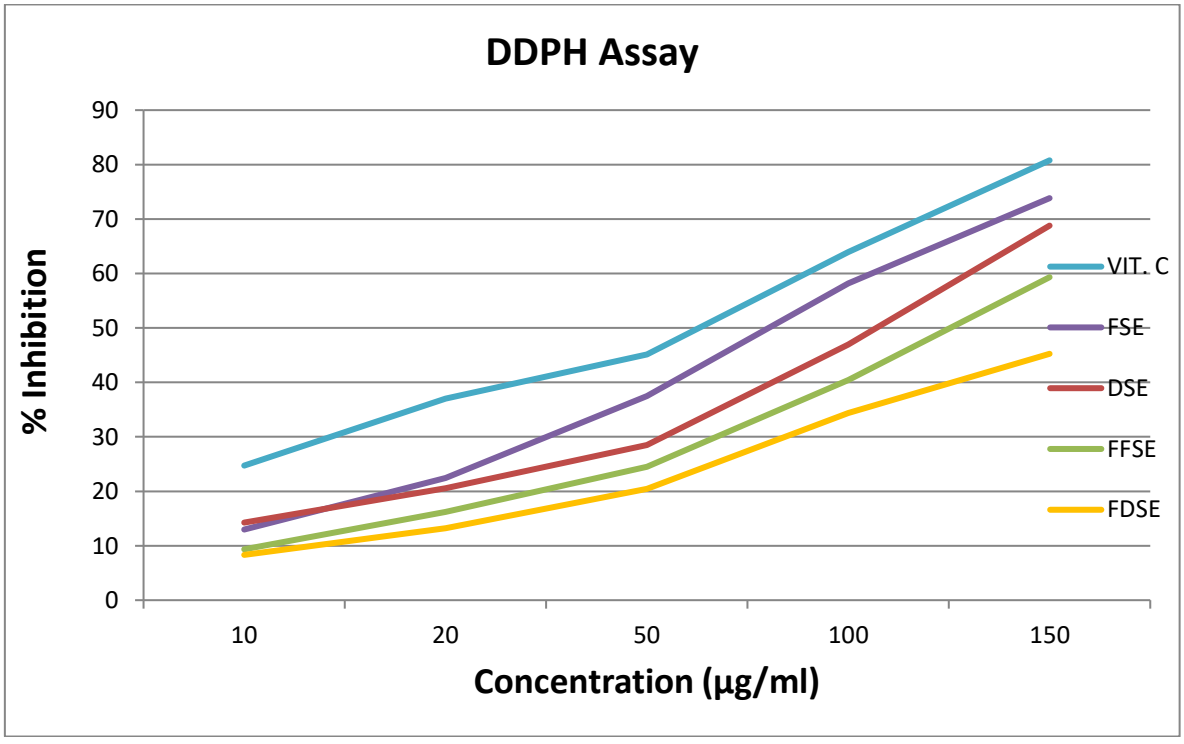

Figure 2. DDPH Assay of DSE, FDSE, FSE and FFSE

Ferric Reducing Antioxidant Power (FRAP) analysis is used to evaluate the capacity of natural antioxidants to donate an electron or hydrogen. In the reducing power analysis, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron (Daniel et al., 2020). The ferric-reducing activities of stem extracts of *C. afer* are presented in Figure 3. All extracts exhibited a similar concentration-dependent activity pattern as given in the DPPH analysis. The reducing power of the extracts followed the order of DSE < FSE < VITC. It can be observed that at a concentration of 20 µg/ml, FSE showed a closer absorbance of 23.31 to the standard (28.42) as compared to DSE with absorbance of 16.85. The antioxidant activity of these extracts could be due to the ability to scavenge specific free radicals. Both FFSE and FDSE showed lower antioxidant activities than their corresponding extracts at all concentrations.

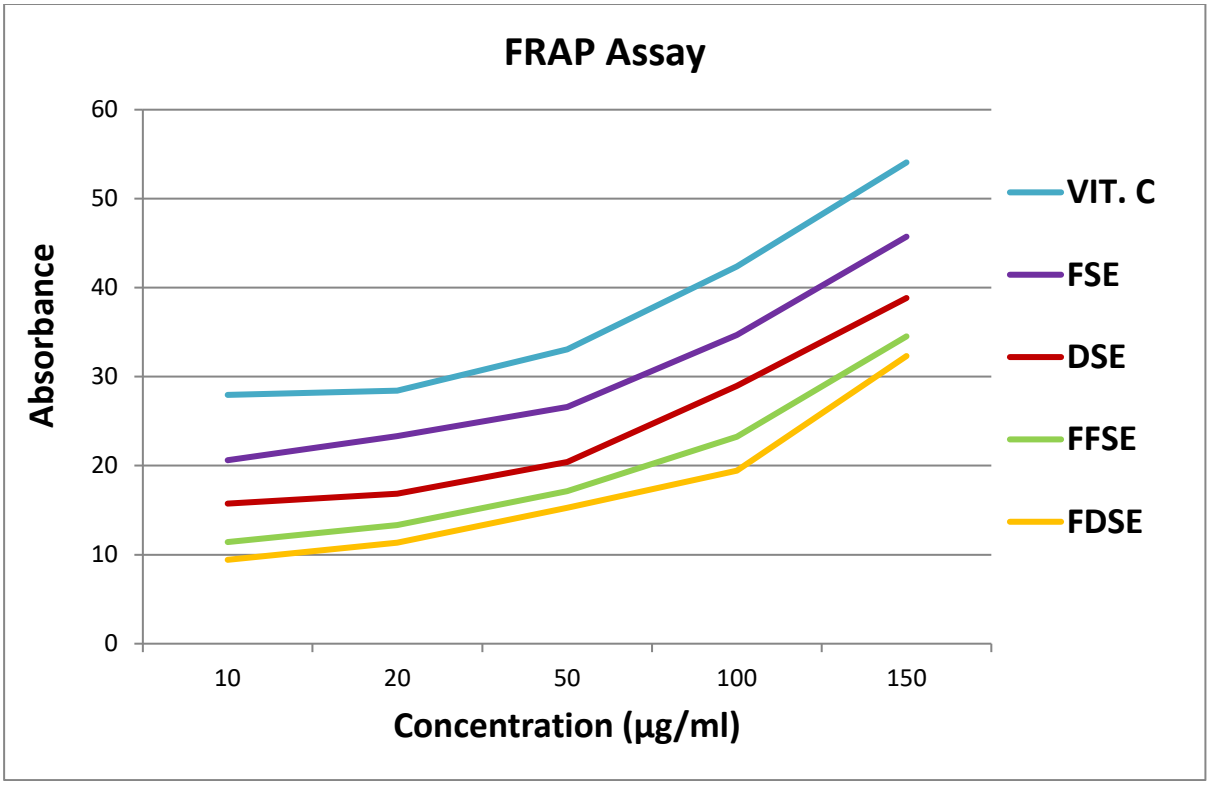

Figure 3. FRAP Assay of DSE, FDSE, FSE and FFSE

ABTS radical scavenging activity was also positively correlated to the concentrations of the stem extracts as seen in DDPH and FRAP analyses. In this analysis, the FSE sample showed higher radical scavenging activity of ABTS than the DSE sample (Figure 4). It was observed that at a concentration of 20 µg/ml, there was a close percentage scavenging ability between DSE and FSE (8.17% and 8.46% respectively). However, there was an observable difference between DSE (31.18%) and FSE (38.39%) at the highest 150 ug/ml concentration. All FDSE and FFSE concentrations showed lower activities than the crude extracts. The noticeable scavenging activity of the plant extracts recorded in this study could be attributed to the presence of phytochemical constituents.

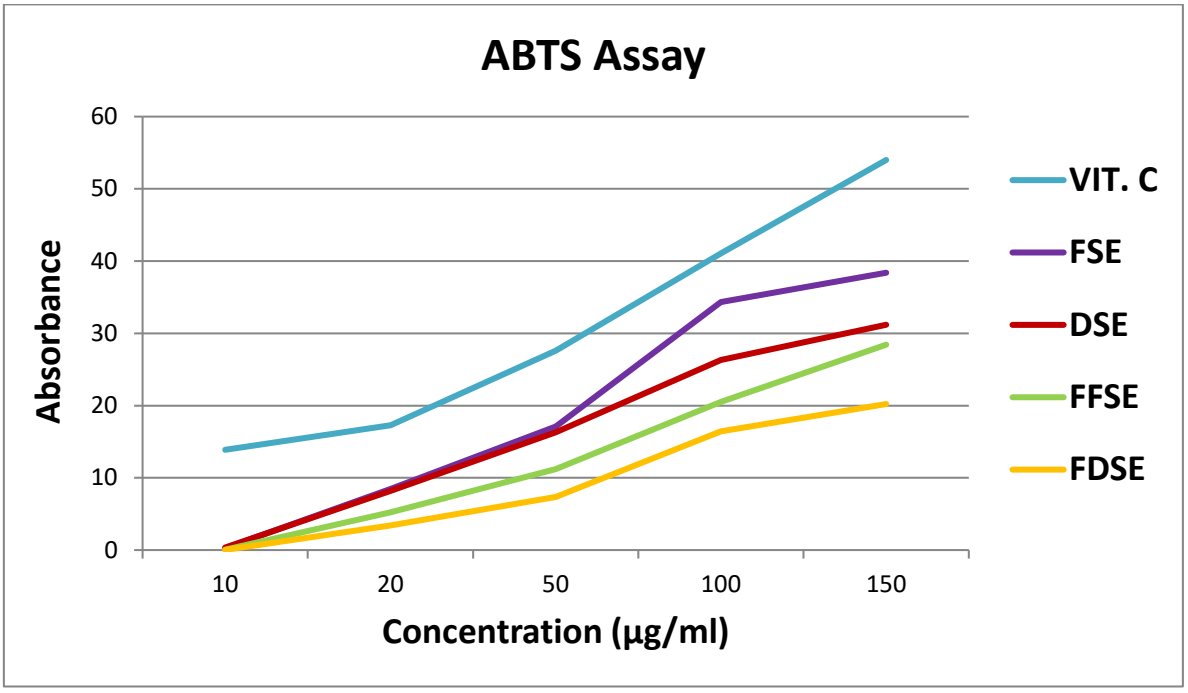

Figure 4. ABTS Assay of DSE, FDSE, FSE and FFSE

Flavonoid Content

The flavonoid contents of FSE and DSE were calculated from the calibration curve (Figure 5) of absorbance against concentration of quercertin. The equation obtained was, y = 0.0003x + 0.0511 (R^2 = 0.9674). From the equation, the content of flavonoid in DSE (153 µg/g) was lesser than that in FSE (186 µg/g).

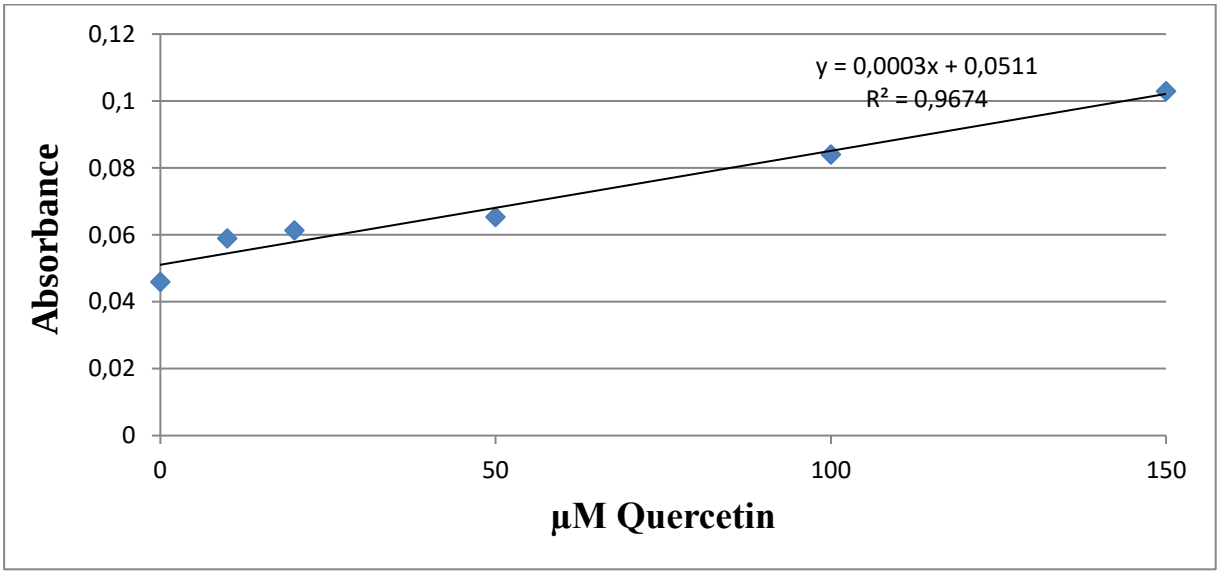

Figure 5. Standard curve for flavonoid content

Phenolic Content

A standard curve using gallic acid as standard was prepared for the estimation of phenolic content of methanolic extracts *Costus afer* stems (Figure 6). The equation obtained between absorbance and amount of gallic acid in µg was y = 0.0004x + 0.058 (R^2 = 0.9458) which shows a linear relation between absorbance and amount of gallic acid. The FSE (435 µg GAE/g) had higher phenol content than DSE (172 µg GAE/g).

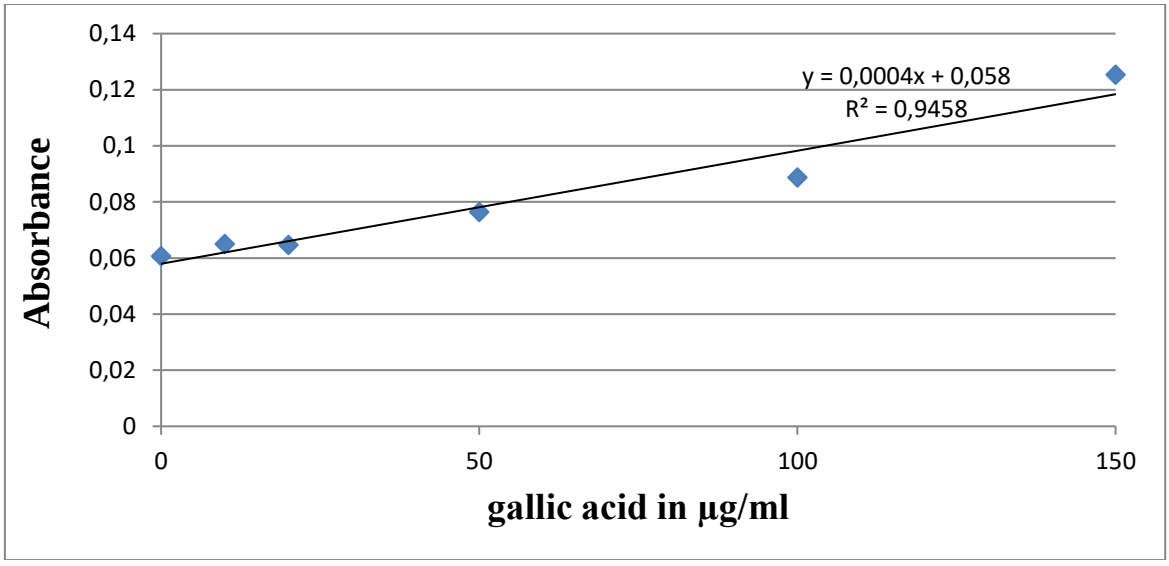

Figure 6. Standard curve for phenolic content

4. CONCLUSION

The levels of antioxidant and antimicrobial activities of FSE and DSE were determined. All the extracts displayed activities in a concentration-dependent manner. The phytochemical compounds detected in the extracts may possibly account for their level of antimicrobial activity (Edema, *et al.*, 2012). Isolation of flavonoid fractions from extracts was done by separation through TLC and CC. The quantity of flavonoids in FSE and DSE was determined. The content of flavonoids in DSE (153 ug/g) was lesser than that in FSE (186 ug/g). The levels of antioxidant and antimicrobial activities of the flavonoid fraction were determined. The observed lower level of antimicrobial activities of FDSE and FFSE with respect to DSE and FSE respectively could be attributed to the absence/very small amount of other phytochemicals like alkaloids in the fractions. Alkaloids are greatly responsible for the level of antimicrobial activities in plant extracts (Daniel and Etukudo, 2019).

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