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Research Paper

Evaluation of Total Phenolic Content, Antioxidant properties, GC-MS and FTIR spectroscopy study of Costus *afer*

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ABSTRACT
Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of their unmatched availability of chemical diversity. This study was aimed at determining the phenolic content, antioxidant activity and elucidation of the bioactive compounds present in butanolfraction of Costus afer root using Fourier transform infrared spectroscopic (FTIR) and Gas chromatography–mass spectrometry (GC-MS). Dried powder of Costus afer root was extracted using maceration method and was fractionated into different fractions. Quantitative phenols were carried out and antioxidant activity was evaluated by using DPPH and FRAP Scavenging assay. The root fraction was further analyzed using GC-MS and FT-IR techniques to identify phytocompounds. Result showed range of phenolic content from 1.73-5.52mg/mL and equally showed high antioxidant activity because of its lower IC50 values and higher percentage inhibitions very near to the controls used. The FTIR spectroscopic studies revealed the presence of these functional groups: amines, phenols, carboxylic acids, alcohols, alkenes, alkanes, and aldehydes in the extract. The results confirmed the presence of 50 compounds while 18 compounds had scientific backings about their biological activities as revealed in this present study. The result suggests that Costus afer has many important secondary metabolites and it has a potential for strong antioxidants.

Keywords: *GC-MS, FTIR, Costus afer, Medicinal Plants, Antioxidant, Phenols, Fraction.*

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I. INTRODUCTION

The use of herbal preparations as a form of therapy dates back to many years ago. Their effectiveness in general management of wellbeing has been attributed to the various phytochemical constituents commonly referred to as secondary metabolites. These compounds as used in phytomedicine can be considered to be the prime foundation of the contemporary allopathic medicines today. The use and dependence on plants as medicines by man has been in existence since time immemorial and man continues to search for plants as drug for a particular disease within his reach. Herbal medicines are safe than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway (Zaidan*et al*., 2005). GC-MS and FT-IR has played an important role in pharmaceutical analysis in recent (Koduru*et al*., 2006) recently, spectroscopy has emerged as one of the major tools for biomedical applications and has made significant progress in the field of clinical evaluation. Research has been carried out on a number of natural tissues using spectroscopic techniques, including FT-IR spectroscopy. Higher plants are sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on the green plants to represent a reservoir of effective chemotherapeutics, which are non-phytotoxic, more systemic and easily biodegradable.

Presently, there has been an increasing interest in the study of traditional plants and their medicinal value in different parts of the world. The medicinal properties of plants have been investigated in the light of recent scientific development throughout the world, due to their strong pharmacological activities, economic viability and low toxicity (Prashant *et al*., 2008). This tremendous interest in plants-derived drugs are mainly due to the current widespread belief that herbal medicine is safer and more reliable than the costly orthodox medicine, many of which may have adverse side effects (Jigma& Sumitra, 2006). Following this, lack of

standardization has placed a limitation in general acceptance of herbal medicines. However, it is impossible to assay for a specific chemical entity when the bioactive ingredient is not known. Additionally, Free radicals have been implicated in the development of a number of disorders, including cancer, neurodegeneration and inflammation, giving rise to studies of antioxidants for the prevention and treatment of diseases (Baba and Malik, 2015). Most of the drugs that are currently available on the market for the treatment of various serious diseases have limited potential because they are expensive and produce detectable side effects.

Costus afer has reportedly been known for differs therapeutic actions traditionally by researchers. *C. afer* is a useful medicinal plant that is highly valued for its ant-idiabetic, anti-inflammatory and anti-anthritic properties in South-East and South-West Nigeria (Omokhua, 2011). In Ogba community of Rivers State, the leaf and stem of *C. afer* when cut and crushed into smaller bits, boiled together with the leaf and bark of Alchorneacordiflora is used for the treatment of hunch bark and malaria. Among the Ikwerre ethnic group in Rivers State, it is applied in various ways. The leaves are reputed to be an effective remedy for fever and malaria when boiled with leave of carica papaya (pawpaw), citrus species (orange) and bark of *Mangifera indica* (mango) (Omokhua, 2011). The stem and juice has traditional use for treatment of cough, measles and malaria in Aluu community of Rivers State. The juice of *C. afer* is extracted and used as an instillation for eye inflammation and defects in Ogoni land, Rivers State. The young and tender leaves when chewed is believed to give strength to the weak and dehydrating patient. An infusion of the inflorescence is taken to treat stomach complaints. A stem decoction (the mashed or chewed stem or the pounded fruit) mixed with sugarcane juices are taken to treat cough, respiratory problem and sore throat [5]. Screening active compounds from plants has led to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases including cancer and alzheimers disease. A majority of the rich diversity of Nigeria medicinal plants is yet to be scientifically evaluated leading to its standardization and *Costus afer* is not left out. However, the present of this study is to evaluate of butanol fraction of *Costus afer* root using spectral analysis, determine its total phenolic contents and antioxidant potential.

II. Materials and Methods

All chemicals and solvents used were of good analytical grades.

Plant Collection

Fresh plant of *Costus afer* root was collected from Nnewi, Anambra State Nigeria. The plants were identified and authenticated by a Botanist in Department of Environmental Biology Federal Polytechnic Nekede Owerri Imo State and deposited in the herbarium of the same institution.

Extraction and Fractionation

The root of *Costus afer* was powdered with a mechanical grinder and passed through Sieve no. 40. Powder of the plant samples was extracted with *n*-butanol by continuous maceration method where it was soaked for 3days. The excess solvent was removed by evaporation using the water bath; the remaining mass of extract was concentrated and dried. The residue was suspended in 50ml water and partitioned successively with *n*-hexane, butanol, ethyl acetate (a total of two aliquots of 100ml each) and soluble residual aqueous fraction yielding respectively the fractions needed (Saeed *et al*., 2012).

Determination of Total Phenolic Content (TPC)

Colorimetric protocol (Ahmed *et al.,* 2014) was used to determine total phenolic content of butanol fraction of *Costus afer* root. In a test tube, 40 µL (1 mg per 1 mL of butanol) of the plant extract (or standard gallic acid solution), 3.16 mL distilled water and 200 µLFolin-Ciocalteu reagent were put and mixed by shaking gently. After an incubation of 8 min, 600 µL sodium carbonate solution was added and mixed. The mixture was incubated at 40 °C for 30 min before recording its absorbance in a spectrophotometer at 765 nm against a blank. The blank contained 40 µL methanol in place of sample. Gallic acid was used as a standard. Its calibration curve was drawn and the total phenolic content was expressed as micrograms per milliliter of gallic acid equivalents (µg/mL of GAE).

DPPH Radical Scavenging Assay

Free radical scavenging activities of the butanol fraction of *Costus afer* root were determined according to the DPPH methods of Ahmed et al. (2014). The stock solution of DPPH radical (24 mg/100 mL in methanol) was prepared. By diluting this solution with butanol, a working solution was prepared to obtain an absorbance of 0.980 ± 0.02 at 517 nm. Stock solution of plant extract/fraction was prepared in butanol with concentration 4 mg/mL. From this, different dilutions (0.2–2.0 mg/mL) were made. The test mixture contained 3 mL DPPH working solution and 100 µL of a sample. The mixture was incubated at 37 °C for 30 min in dark. Absorbance of each sample was recorded at 517 nm. For a negative control, 100 µL of butanol was added in place of plant sample. Ascorbic acid was used as a positive control. Inhibition curves were made and IC50 value were calculated for all samples. The free radical scavenging activity of each sample was calculated by using the formula:

% Scavenging Activity = 100[(Ac – As)/Ac] (1)

where Ac and As are absorbances of negative control and sample, respectively.

Frap assay

The FRAP assay was used to estimate the reducing capacity of root of *Costus afer* fraction according to the method of Oyaizu (2008). The FRAP reagent contained 2.5ml of TPTZ in dilute hydrochloric acid, 20ml of ferric chloride and 25ml acetate buffer was prepared freshly and warmed to 35ºC. FRAP reagent 1.5ml and sample solution 50µl at different concentration was incubated at 37ºC for 10mins and absorbance was recorded at 593nm. Ferrous sulphate was used as a standard. FRAP value was expressed as mmoles/100 on dry weight basis using the calibration curve of Fe2+.

Gas Chromatography–Mass Spectrometry (GC-MS) ANALYSIS

Methanol fraction of *Costus afer* were analyzed with the help of GC-MS analyzer (GC-MS-QP 2010 plus Shimadzu, Japan). The carrier gas helium (99.999 %) was used at a flow rate of 1 ml per min in split mode (10:1) v/v. Methanol and chloroform extracts (8 µl) were injected into the column at 250 °C injector temperature. Temperature of oven started at 70 °C and held for 5 min. It was then raised at the rate of 10 °C per min to 280 °C without holding. Analyze was allowed for 6 min at programmed rate of 5 °C per min. Temperature of ion sources was maintained at 200 °C. The injector temperature was set at 250 °C and detector temperature was set at 250 °C. The mass spectrum of compounds present in samples was obtained by electron ionization at 70 eV and detector operates in scan mode 50 to 600 Da atomic units. The MS Table was generated through ACQ mode scan within 0.5 seconds of scan interval at the speed of 666 and fragments from 30 to 350 Da was maintained. Total running was 21 minutes (Odo*et al*., 2017).

Identification of Compounds

Identification of the compounds were based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. The spectrum of unknown components was compared with the spectrum of the known components stored in the NIST library.

FTIR Analysis

Buck scientific M530 USA FTIR was used for the analysis. This instrument was equipped with a detector of deuterated triglycine sulphate and beam splitter of potassium bromide. The software of the Gram A1 was used to obtain the spectra and to manipulate them. An approximately of 1.0g of samples, 0.5ml of nujol was added, they were mixed properly and placed on a the salt pellet.. During measurement, FTIR spectra was obtained at frequency regions of 4,000 – 600 cm-1 and co-added at 32 scans and at 4 cm-1 resolution. FTIR spectra were displayed as transmitter values (Odo*et al*., 2017).

III. Result and Discussion

Total Phenolic Content

Table 1: Total Phenolic contents in *Costus afer* root butanol fraction

PHENOLIC CONTENT	
Concentration of extract (mg/ml)	**Concentration of Phenolic content (mg/ml)**
10	1.734 ± 0.00^{a}
20	2.520 ± 0.00^{b}
40	3.407 ± 0.00^{d}
80	3.226 ± 0.00^{c}
Gallic acid (20mg/ml)	5.524 ± 0.00^{e}

n = 2. Results are expressed in mean ± standard deviation with mean values with the different letters as superscripts across columns are considered significant ($p < 0.05$) while mean values with the same letters as superscripts acrosscolumns are considered non-significant ($p > 0.05$).

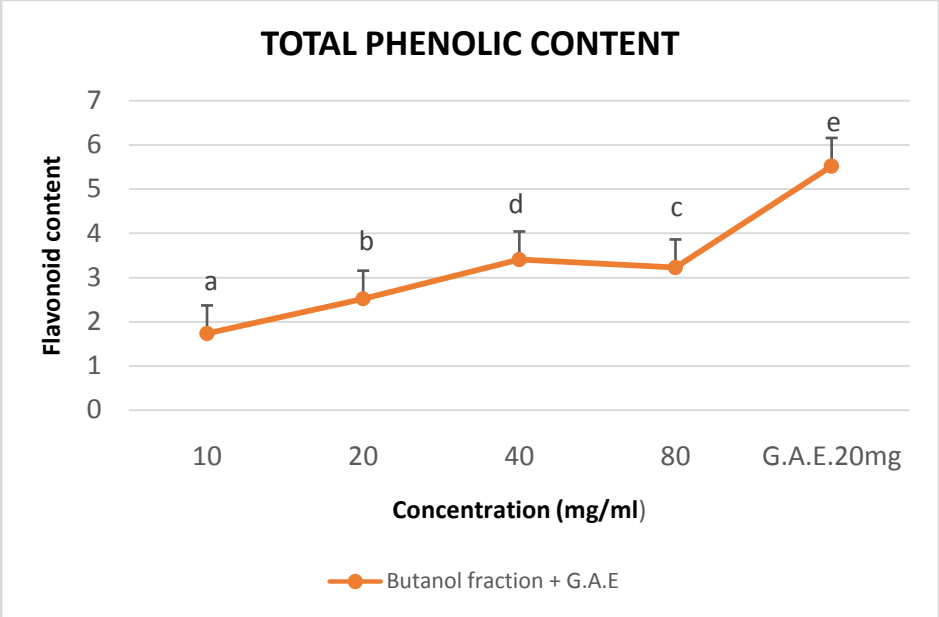

Fig (1): Graph showing TPC

DPPH Scavenging Assay

Table 2: DPPH scavenging activity of *Costus afer* root butanol fraction

DPPH SCAVENGING ACTIVITY		
Concentration of extract (mg/ml)	**% Inhibitions**	**IC50 (µg/ml)**
10	92.99 ± 0.26^{a}	-75.182
20	94.05 ± 0.15^{bc}	-65.957
40	94.34 ± 0.11^{c}	-47.507
80	93.87 ± 0.11^{b}	-10.607
BHT	98.50 ± 0.00^{d}	

n = 2. Results are expressed in mean ± standard deviation with mean values with the different letters as superscripts acrosscolumns are considered significant ($p < 0.05$) while mean values with the same letters as superscripts acrosscolumns are considered non-significant ($p > 0.05$).

FRAP Scavenging activity

Table 3: FRAP Scavenging activity in *Costus afer* root butanol fraction

FRAP SCAVENGING ACTIVITY		
Concentration of extract (mg/ml)	**% Inhibitions**	**IC50 (µg/ml)**
10	52.95 ± 0.01^{a}	-7.223
20	59.16 ± 0.00^{b}	-5.284
40	61.99 ± 0.02^{c}	-1.406
80	65.64 ± 0.01^{d}	6.348
Gallic acid (10mg/ml)	70.70 ± 0.01^{e}	
Gallic acid (20mg/ml)	81.40 ± 0.01^{f}	

n = 2. Results are expressed in mean ± standard deviation with mean values with the different letters as superscripts across columns are considered significant ($p < 0.05$) while mean values with the same letters as superscripts acrosscolumns are considered non-significant ($p > 0.05$).

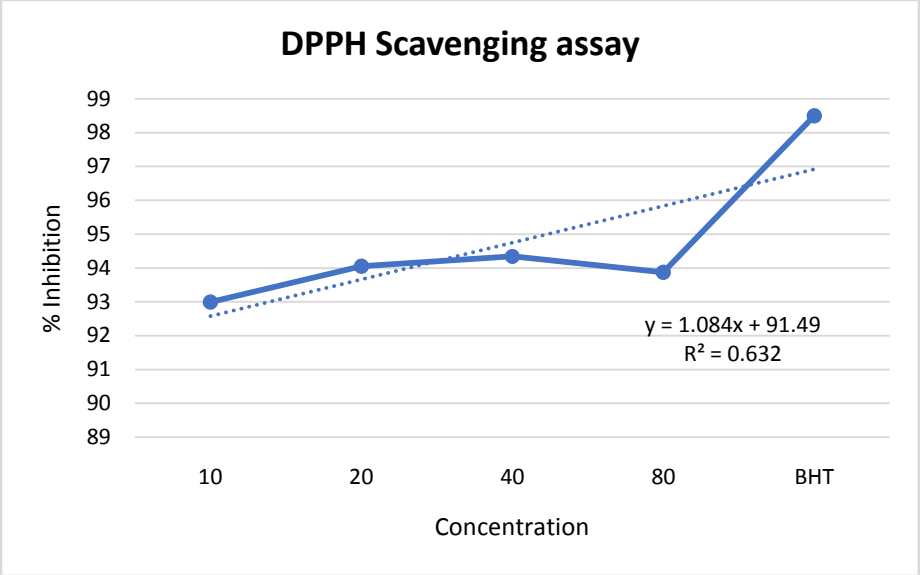

Fig (II): Graph Showing DPPH scavenging activity

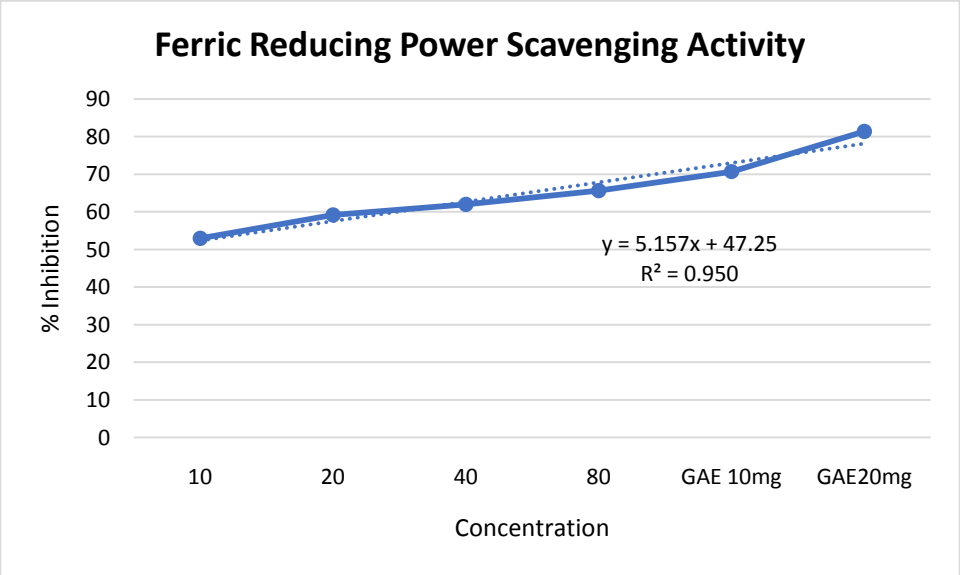

Fig (III): Graph Showing FRAP scavenging activity

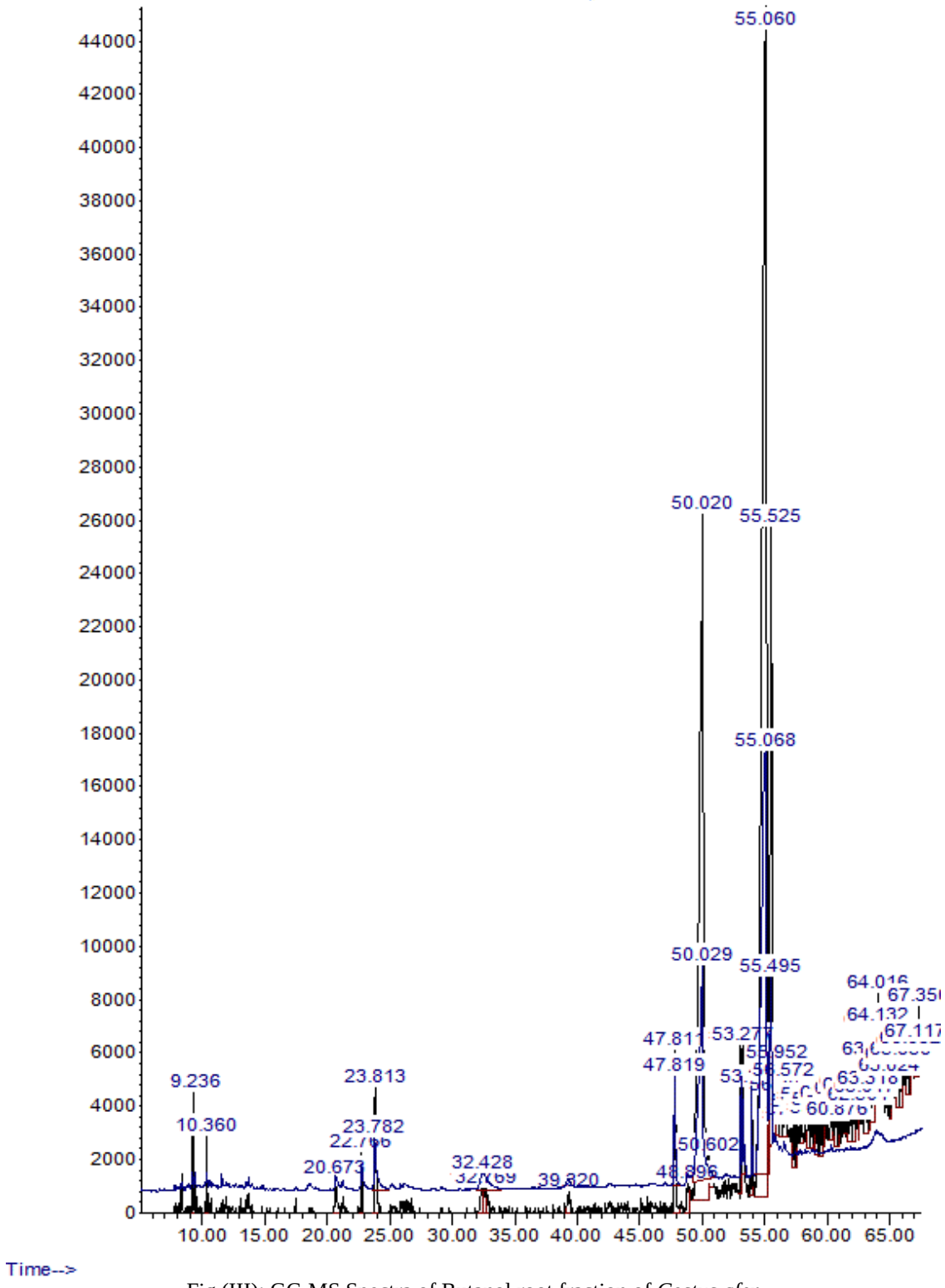

Fig (III): GC-MS Spectra of Butanol root fraction of *Costus afer*

GC-MS Analysis results with their pharmacological activities

S/N	Retention Time	Name of Compound	Molecular formula	Molecular weight	Area %	Pharmacological activities	References
1.	9.236	2H-Pyran, 2-(2,5-hexadiynyloxy)tetrahydro-	$C_{11}H_{14}O_2$	178.28	0.79	None Reported yet	
2.	10.360	2-Propenenitrile	C_3H_3N	53.06	0.54	None Reported yet	
3.	20.673	Difluoramine	F_2HN	53.0114	0.46	None Reported yet	
4.	22.766	Cyclopropanecarbonitrile	C_4H_5N	67.09	0.58	None Reported yet	
5.	23.813	3-Penten-1-yne	C_5H_6	66.101	1.66	None Reported yet	
6.	32.342	Carbonyl sulfide	COS	60.075	0.52	None Reported yet	
7.	32.536	2-Propenenitrile	C_3H_3N	53.06	0.46	None Reported yet	
8.	32.769	Thiirane	C_2H_4S	60.118	0.30	Antimicrobial and cytotoxic activities	(Asif, 2014)
9.	39.320	2-Propenenitrile	C_3H_3N	53.06	0.41	None Reported yet	
10.	47.811	Hexanoic acid	$C_6H_{12}O_2$	116.15	1.62	Antimicrobial and cytotoxic activities	(Researchgate, n.d)
11.	48.896	Hydrazine	H_4N_2	32.04	0.63	Antioxidant	(Zhong et al., 2010)
12.	50.020	6-bromo-Hexanoic acid	$C_6H_{11}BrO_2$	195.054	22.48	None Reported yet	
13.	50.602	1-Propanol	C_3H_8O	60.095	0.44	Antiviral	(VAH, 2021)
14	53.083	4-methyl- 1-Butene	$\mathbf{C_6H_{12}}$	84.162	1.11	None Reported yet	
15	53.277	10-Azido-1-decanethiol	$C_{10}H_{21}N_3S$	215.359	0.78	None Reported yet	
16	53.975	3-Pyrrolidinol	C_4H_9NO	87.120	0.86	Antibacterial, antifungal, anticancer and anticonvulsant.	(Hosseinzadeh et al., 2018)
17	55.060	5-Hexenoic acid	$C_6H_{10}O_2$	114.142	43.89	None Reported yet	
18	55.525	5-Hexenoic acid	$C_6H_{10}O_2$	114.142	7.21	None Reported yet	
19	55.952	Propionic acid 3-tetrazol-1-yl-2-Amino-1,3-propanediol	$C_3H_6O_2$	74.0785	0.51	Antibacterial	(Peh et al., 2020)
20	56.301	1,5-Hexadiene	C_6H_{10}	82.143	0.46	None Reported yet	
21	56.572	Aminoacetonitrile	$C_2H_4N_2$	56.066	0.71	Antihelminthic	(Ducray et al., 2008)
22	57.309	1-Methyl-3-butenyl 3-methyl-3-hydr oxybutyl ether	$C_{10}H_{20}O_2$	172.260	0.31	None Reported yet	
23	57.309	Propanal	C_3H_6O	58.0791	0.31	Antimicrobial	(Lamba, 2007)
24	57.929	4-Cyclopentene-1,3-diol	$C_5H_8O_2$	100.116	0.38	Anticancer agent	(Google, n.d)

25	58.472	Thiirane	C_2H_4S	60.118	0.51	Antimicrobial and cytotoxic activities	(Asif, 2014)
26	58.743	Hydrazine	H_4N_2	32.04	0.49	Antioxidant	(Zhong et al., 2010)
27	59.131	1-Pentanol	$C_5H_{12}O$	88.148	0.26	Antibacterial	(Garrido et al., 2020)
28	59.286	2,4-Pentadienenitrile	C_5H_5N	79.099	0.31	None Reported yet	
29	59.519	Urea	CH_4N_2O	60.055	0.39	Antioxidant	(Sudhamani et al., 2019; Clinical Trials Gov, n.d)
30	59.712	Acetic acid	$C_2H_4O_2$	60.05	0.40	Antibacterial	(Zinn &Bockmühl, 2020)
31	60.372	Isobutylamine	$C_4H_{11}N$	73.136	0.43	None Reported yet	
32	60.682	1,3-Butadiene	C_4H_6	54.090	0.41	Antibacterial and antifungal	(Aydinli et al., 2020; Tahir et al., 2017)
33	60.876	Methane	CH_4	16.042	0.26	Antioxidant, anti-inflammatory and antiapoptotic	(Jia et al., 2018)
34	61.496	Urea	CH_4N_2O	60.055	0.32	Antioxidant	(Sudhamani et al., 2019; Clinical Trials Gov, n.d)
35	61.845	Pentadecylamine	$C_{15}H_{33}N$	227.429	0.65	None Reported yet	
36	62.116	Isobutylamine	$C_4H_{11}N$	73.136	0.42	None Reported yet	
37	62.504	2-Methylenecyclohexanol	$C_7H_{12}O$	112.169	0.26	None Reported yet	
38	63.047	oxime 1-Butene	$C_{10}H_{11}NO$	161.20	0.53	None Reported yet	
39	63.318	2-Propanamine	C_3H_9N	59.110	0.48	None Reported yet	
40	63.706	Pentanoic acid	$C_5H_{10}O_2$	102.1317	1.10	None Reported yet	
41	63.861	D-erythro-Pentose	$C_5H_{10}O4$	134.130	0.72	None Reported yet	
42	64.016	Oxirane	C_2H_4O	44.052	0.47	Antibacterial	(Thirunarayanan, 2014; Yusuf et al., 2020)
43	64.132	Isobutylamine	$C_4H_{11}N$	73.136	0.44	None Reported yet	
44	65.024	Cyclopropane	C_3H_6	42.079	0.27	Insecticidal, antifungal, herbicidal, antimicrobial, antibiotic, antibacterial, antitumor and antiviral activities	(Kumar, 2014)
45	65.566	D-Mannopyranose	$C_5H_{10}O_4$	134.13	0.39	None Reported	

		1,2,3,4-Cyclopentanetetrol				yet	
46	65.838	1,2,3,4-Cyclopentanetetrol	$C_5H_{10}O_4$	134.130	0.64	None Reported yet	
47	66.381	2-Penten-1-ol	$C_5H_{10}O$	86.132	0.51	None Reported yet	
48	66.652	Nitric acid	HNO_3	63.012	0.49	None Reported yet	
49	67.117	2-Octyn-1-ol, 1,6-dichloro- Morpholine, 4-methyl-, 4-oxide	$C_8H_{14}O$	126.196	0.42	None Reported yet	
50	67.350	10-Undecyn-1-ol 1,4,9-Decatriene	$C_{11}H_{20}O$	168.275	0.58	Antifungal	(AlfaAesar, 2022)

Functional groups identified in FT-IR analysis

Extracts	Group	Compound class
881	C=C bending	Alkene
1311, 1372	C-O Stretching, S=O Stretching	Aromatic ester, Sulfonamide
1668, 1942	C=C Stretching, C-H bending	Alkene, Aromatic
2155, 2510, 2900	S-C-N Stretching, N=N=N Stretching, S-H Stretching, C-H stretching	Thiocyonate, Azide, Thiol, Alkane
3251, 3405, 3806	O-H Stretching	Alcohol

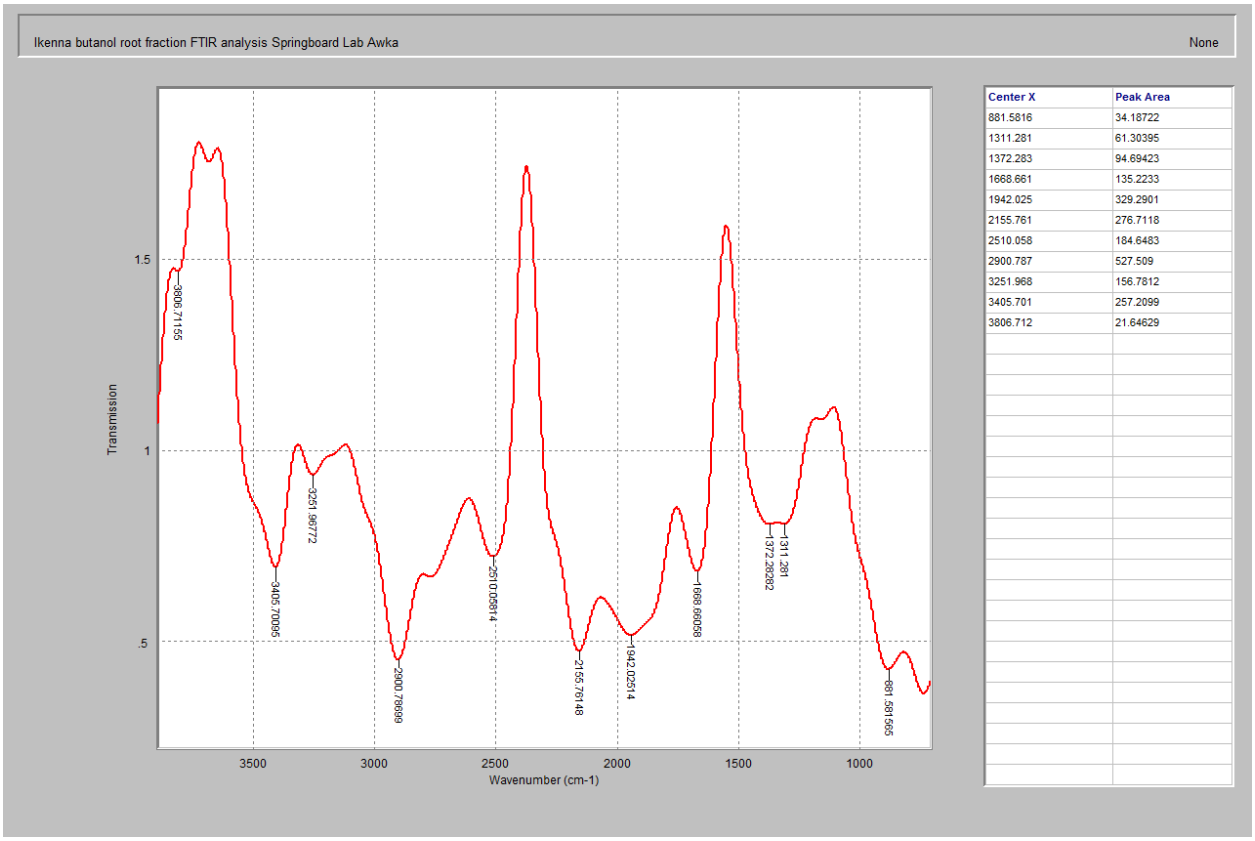

Fig (IV): FT-IR Spectra of Butanol root fraction of *Costus afer*

Discovery and development of new antioxidant drugs is among the most exciting areas of pharmacological research. Oxygen is a vital element of aerobic life, but under certain conditions it can seriously influence our health by formation of reactive oxygen species (free radicals) leading to some potentially dangerous diseases, like coronary heart disease, diabetes, atherosclerosis, neurodegenerative disorders (AD & Dementia), cancer, immune-suppression, ageing and ulcer (Ayaz *et al*., 2014). The bioactive constituents in plants are ubiquitously distributed in various tissues of plants (Ezealigo*et al*., 2020).

Most common free radicals include hydroxyl, nitric oxide, superoxide & lipid peroxyl, whereas non-free radicals primarily include singlet oxygen and hydrogen peroxide (Ayaz *et al*., 2014). Nevertheless, approximately all living organisms are protected from free radicals attack by defense system, such as a protective antioxidant system that diminish the rate of free radical formation along with another system which create chain-breaking antioxidants to scavenge & stabilize free radicals. However, when the rate of free radical generation exceeds the capacity of defense mechanisms, extensive tissue injury results (Ayaz *et al*., 2014). Consequently, drugs with free radical scavenging abilities are useful for the prevention and therapy of these diseases. Antioxidant compounds are known to show their biochemical effects via several mechanisms, including hindrance of chain initiation, chelation of metal ions, breakdown of peroxides, sustained hydrogen abstraction, reductive ability and radical scavenging. Hence, numerous methods are proposed to assess the antioxidant activity. DPPH is an extensively used model system to evaluate the free radical scavenging potential of drugs. DPPH radicals are scavenged by antioxidants through the donation of hydrogen, thus forming reduced DPPH-H, which change the color from purple to yellow following reduction and is quantified by analyzing absorbance at wavelength 517 nm.

Phenolics are a class of antioxidant compounds which function as free radical terminators (Shahidi and Wanasundara, 1992). Previous reports indicate that the free radicals scavenging efficiency of phenolics is dependent on their molecular weight, presence of aromatic rings and nature of OH group's substitution (Ayaz *et al*., 2014). Table 1 shows extraction yield of phenolics (mg GAE/g of sample) indicating that butanol root fraction of *Costus* afer expressed good concentrations of phenolics. Results of DPPH and FRAP scavenging activities well correlates with phenolic content and might be attributed to presence of high molecular phenolics in addition to flavonoids in these fractions of plant. The inhibitory values (IC50) were of ranges 0.485-6.355 µg/ml fractions in FRAP free radical scavenging assay and 0.747-3.573 µg/ml for DPPH assay which exhibited good activity comparable to their controls Gallic acid and BHT respectively. Our findings indicate that *Costus afer* is enriched with antioxidant compounds and show its possible effectiveness in the management of free radicals induced disorders especially neurodegenerative diseases.

Phytochemicals have been recognized as the basis for the traditional herbal medicine. The presence of various types of phytochemicals in plant has implicated in the health promoting properties of medicinal plants. The previous study in 2018 by Nnaoma et al has revealed that root of *Costus afer* contains anthraquinones, cardiac glycosides tannins, flavonoids, and phlobatanins. Results of the GC-MS analysis of *Costus afer n*-butanol root fraction shows 50 compounds were present in the plant root analyzed. The functional groups present in *Costus afer n*-butanol root fraction are; Alcohols, alkene, carboxylic acid, Aromatic ester, sulfonamide, thiocyanate azide, and thiol. They were confirmed by the FTIR Spectra which revealed the presence of these groups; O-H, C=C, C-O, S=O, C-H, S-C-N, N=N=N, S-H, and C-H, these compounds present has been reported by other studies to be responsible for plant's biological activities.

The study identified 50 compounds from the *Costus afer n*-butanol root fraction, showing the most abundant from the plant which is 5-Hexenoic acid was present with percentage peak of 43.89% and retention time 55.060 forming the major constituents in the root followed by 6-bromo-Hexanoic acid with percentage peak 22.48% and retention time 55.020 but no report has shown any pharmacological activity of these major constituents. Nevertheless, 18 out of 50 identified compunds showed some wide range of pharmacological activities. Among the compounds are; Thiirane (RT: 32.769, peak %:0.30), Hexanoic acid (RT: 47.811, peak %: 1.62), Propanal (RT:57.309, peak %:0.31) has been reported to exhibit some antimicrobial and cytotoxic activities (Asif, 2014; Lamba, 2007; Researchgate, n.d), Hydrazine (RT: 48.896, peak %:0.63) and Urea (RT: 59.519, peak %:0.39) possess antioxidant as confirmed by Zhong *et al* (2010), Sudhamani et al., (2019) & Clinical Trials Gov (n.d), 1-Propanol (RT: 50.602, peak%:0.44) has been reported to possess antiviral properties (VAH, 2021), 3-Pyrrolidinol (RT: 53.975, peak %:0.86) equally has been confirmed to have antibacterial, antifungal, anticancer and anticonvulsant properties (Hosseinzadeh*et al*., 2018), Aminoacetonitrile (RT: 56.57, peak %: 0.71) has been studied to have antihelminthic effect (Ducray*et al*., 2008), 1-Pentanol (RT: 59.131, peak %:0.26), Oxirrane (RT: 64.016, peak %: 0.470), Propionic acid and Acetic acid (RT: 59.712, peak %: 0.40) has been reported also to have specifically antibacterial activity (Garrido *et al*., 2020; Peh*et* al., 2020; Thirunarayanan, 2014; Yusuf et al., 2020; Zinn &Bockmühl, 2020), 4-Cyclopentene-1,3-diol (RT: 57.929, peak %:0.38) possess anticancer activity according a reviewed literature by google (n.d), 1,3-Butadiene (RT: 60.682, peak %: 0.41) possess specifically antibacterial and antifungal activity (Aydinli*et al*., 2020; Tahir *et al*., 2017), Methane (RT: 60.876, peak %: 0.26) according to previous studies has shown to have antioxidant, anti-inflammatory and antiapoptotic properties (Jia *et al*., 2018) and Cyclopropane (RT: 65.024, peak %:0.27) has shown to have insecticidal, antifungal, herbicidal, antimicrobial, antibiotic, antibacterial, antitumor and antiviral activities according to Kumar (2014).

IV. Conclusion

Costus afer n-butanol root fraction is endowed with a lot of bioactive compounds with known medicinal application. Hence, the wide use of the plant in traditional medicine in treating various diseases has been a growing trend and with the development of new technologies some of the agents which failed earlier clinical studies are stimulating renewed interest. In the light of our findings, it can be concluded that the fractions of our plant screened herein exhibited good antioxidant potential and can be related to presence of high molecular weight phenolics. The plant has also showed inhibitory activity using FRAP and DPPH assay in dose-dependent way.

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