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Antioxidant, bioactivity and cancer selective cytotoxicity potency of *Eulophia gracilis* successive extracts on selected cancer cells: An *in vitro* model

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ABSTRACT

The metabolisms of many efficient chemotherapeutics are usually accompanied with health challenged toxic side effects and hence the need for more anticancer agents with little or no toxic consequences. The screening of medicinal plants with known ethnopharmacology can be an option to discover new anticancer agents with mild side effects. *Eulophia gracilis* is a medicinal orchid shown to be safe and used traditionally in the treatment of tumors and as an aphrodisiac. This study investigated the antioxidant potential, bioactivity and selective cytotoxicity of successive extracts of *Eulophia gracilis* on cancer cells. The phenolic content, flavonoid content and in vitro antioxidant assays were carried out on successive extracts of the plant. Brine shrimp lethality biological assay and cytotoxicity using MTT assay with four cancer cell lines of human origin: HEp-2 (laryngeal cancer cells), Rd (rhabdomyosarcoma), MCF-7 (breast cancer cell), and HeLa (cervical carcinoma) together with normal kidney epithelial cells (vero) from an African green monkey were determined. Dichloromethane (DCM) fraction among successive fractions has the highest phenolic and flavonoid contents of 159.04 ± 1.24 µg GAE/mg and 501.94± 33.39 µgQE/mg, respectively and correspondingly has the highest DPPH and ABTS radical scavenging effects. The study showed that all the fractions have activities on the brine shrimps and selected cancer cell lines under study. DCM fraction (LC50 =13.8545 µg/mL) was most active relative to other fractions of extracts in the Brine shrimp assay. Moreover, DCM fraction elicited more potent cytotoxicity (8. 65µg/mL on Rd, 0.79 µg/mL on Hep-2c) on RD and HEp-2 than other three fractions. Overall, *Eulophia gracilis* plant possesses antioxidant activity and its DCM fraction is selective to all the cancer cell lines used and has the highest selective index among the fractions for RD, HEp-2, and MCF-7 cell lines.

Keywords: *Eulophia gracilis,* chemotherapeutic efficiency, bioactivity, antioxidant potential, selective cytotoxicity

INTRODUCTION

Cancer is ranked the second leading cause of global death with global burden risen to about 20 million new cases and 9.7 million deaths in 2022 (Bray et al., 2024). In the USA alone, there was a projection of 1,958,310 new cancer cases and 609,820 cancer deaths in 2023 (Siegel et al., 2023). Cancer treatment and management through synthetic chemotherapy poses challenges due to toxic side effects of the cytotoxic agents (Liu et al., 2020). Many of the synthetic anticancer drugs offer off target toxicity effect on rapidly proliferating normal cells such as reproductive cells and major organs like liver and kidney that hosted their metabolizing enzymes (Torri et al., 2021). The resultant menace of the toxic effects of chemotherapy partly shifted the attention of cancer patients from orthodox medicine to traditional healthcare system. The interest of the populace in the use of traditional medicine is increasing especially in African continent due to safety purpose in addition to being the reliable source of active principle used in modern day marketed drugs (Veeresham, 2012). Medicinal plants and their products are reliable sources of medicines where an appreciable percentage of anticancer and anti-infective approved agents are originated from plants (Cragg & Newman, 2005; David & Gordon, 2012; Elujoba et al., 2005).

Figure 1. *Eulophia gracilis* plant and its pseudobulb (06/05/2017) (Source: Authors' own elaboration)

Presently, global demand for natural products has led to an increase in research on many angiosperms as they are reliable source of safe pharmaceutical products (Ogbole et al., 2018) and *Orchidaceae* is one of the largest families of angiosperm. Orchids are primarily cultivated as ornamentals but also offer medicinal values in traditional medicine across the globe (Ho & Chen, 2003; Hossain, 2011). *Eulophia* genus belong to the family of *Orchidaceae*. Most studies have revealed antioxidant activities of several *Eulophia* species (Kumar et al., 2013; Tatiya et al., 2013). Several *Eulophia* species have been reported to possess cytotoxicity and antiproliferative potential on human cancer cell lines (Bhatt et al., 2018; Schuster et al., 2017). *Eulophia gracilis* is an angiosperm that belong to the genius *Eulophia* of *Orchidaceae* family (Hossain, 2011). It is an important medicinal orchid used to treat tumors, liver disorders, as an aphrodisiac and blood related diseases (Ola & Odunola, 2023a, 2023b). It was reported to contain phytochemicals of pharmacological importance such as glycoside, alkaloid, tannins, phlobatanins and flavonoid (Ola, 2017). Despite the wide medicinal reports on *Eulophia* species there is a paucity of information on its cytotoxicity potency on cancer cells and chemotherapeutic potential. This work therefore investigated the antioxidant potential, brine shrimp lethality (BSL) potency, and cytoselective capability of *Eulophia gracilis* pseudobulb fractions on selected cancer cell lines of clinical importance.

MATERIALS AND METHODS

Collection and Authentication of the Plant

Eulophia gracilis plant (**Figure 1**) was harvested at location between Olugbile Village and Okansa Village of longitude (N07^{0}54^{l}36.0-N07^{0}54^{l}37.2) and latitude (E004^{0}00^{l}06.8-E004^{0}00^{l}07.0) at elevation ranging from 337 m to 338 m, Oyo, Oyo state in Nigeria. It was identified and authenticated by a plant taxonomist at the herbarium section of the department of botany, University of Ibadan with herbarium number 22528.

Preparation and Extraction of the Plant

The pseudobulbs of the plants were detached and rinsed in water, sliced to pieces and then dried under shed. The dried pseudobulbs were pulverized into powder using an electric grinder (SFP 2210). The powder (200 g) was extracted under stirring with 800 milliliters of n-Hexane at room temperature for 72 hours and filtered by Buchner funnel. The extraction process was repeated twice with more n-Hexane and filtrate was concentrated under vacuum to obtain yellow residue.

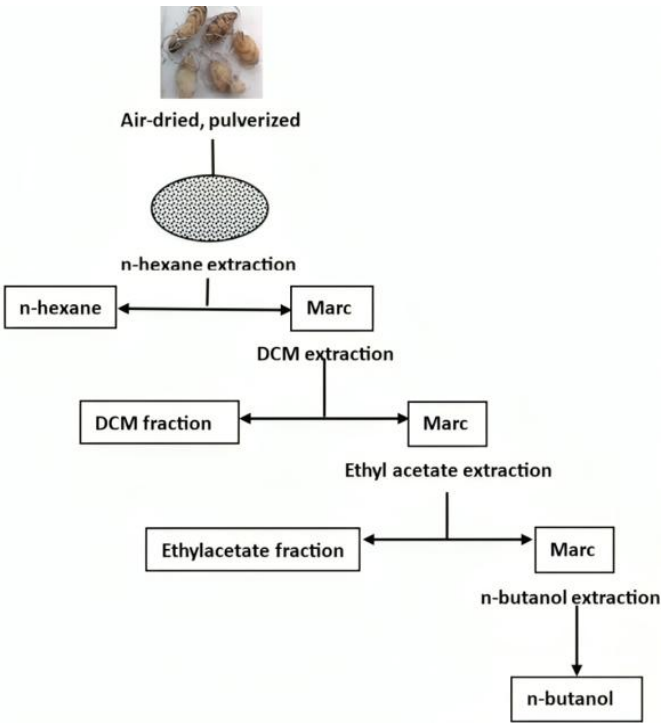


Figure 2. Schematic process for preparation and extraction of the *Eulophia gracilis* plant (Source: Authors' own elaboration)

The Marc was further extracted sequentially with dichloromethane, ethyl acetate and butanol following the same order, as shown in **Figure 2**. The solid mass obtained from each fraction was refrigerated at -4 °C until analysis.

Evaluation of Total Phenolic

Total phenolic content of four fractions of the plant were evaluated with the Folin-Ciocalteau reagent following procedure of Spanos and Wrolstad (1990) as slightly modified by Lister and Wilson (2001). The test samples were performed in triplicate and the mean values were used for plotting of graph. Results are presented in micrograms of gallic acid (GA) equivalents in one milligram of dry weight of extract (µg GAE/mg).

Determination of Total Flavonoid

Total flavonoid content (TFC) was estimated following the method described by Nickavar et al. (2007). TFC for each extract fraction was measured in micrograms of quercetin equivalents in one milligram of extract (µg QE/mg).

In Vitro Antioxidant Activity Assays

The abilities of the four successive fractions from plant pseudobulb to scavenge free radical of 2,2-diphenyl-1picryl hydrazyl (DPPH) were assessed as described by Nickavar et al. (2007). The percetage radical scavenging was calculated, as follows:

$$DPPH\ scavenge\ (\%) = \frac{(A_{cont.} - A_{sample})}{A_{cont.}} \times 100. \quad (1)$$

Absorbance of control and sample at 518 nm are A_{cont} and A_{sample}, respectively. Ascorbic acid serves as positive control.

Table 1. Total phenolic and total flavonoid content of successive fractions of *Eulophia gracilis* extract

Fraction	Total phenolic content in µg GAE/mg	Total flavonoid content in µg QE/mg
n-Hexane	55.56 ± 0.93^{bcd}	442.78± 19.19^{bcd}
DCM	159.04 ± 1.24^{ad}	501.94± 33.39^{acd}
Ethylacetate	158.65 ± 0.17^{ad}	271.56±6.74^{abd}
Butanol	113.74 ± 2.26acd	115.00 ± 5.46^{abc}

Note. All data are presented as the mean (n = 3) ± standard deviation in the same column & the a, b, c, d, and e letters indicate significant differences from means of n-Hexane, DCM, ethylacetate, and butanol fractions respectively ($p < 0.01$)

Hydroxyl Radical Scavenging Assay

The hydroxyl radical was determined as highlighted by Halliwell et al. (1987). Butylated hydroxyltoluene (BHT) and GA (50-800 µg/ml) were used to serve as positive controls. The hydroxyl radicals scavenging activity is calculated, as follows:

$$Inhibition\ (\%) = \left(1 - {}^{A}/_{A_0}\right) \times\ 100. \quad (2)$$

A represents the sample absorbance and A_0 stands for absorbance of negative control at 532 nm.

Determination of ABTS Radical Cation Scavenging Activity

ABTS radical scavenging activity was carried out following the highlight of Re et al. (1999) where each fraction was compared to standard ascorbic acid and BHT. The experiment is in triplicate and scavenging capacity of the free radical was expressed by IC_{50}.

Nitric Oxide Scavenging Activity

The evaluation of nitric oxide scavenging activity of *Eulophia gracicilis* was undertaken by the method of Vaijanathappa et al. (2008), as follows:

$$Percent\ inhibition\ of\ nitric\ oxide = \frac{Abs\ control - abs\ sample}{Abs\ control} \times\ 100. \quad (3)$$

Hydrogen Peroxide Scavenging Activity

The scavenging of hydrogen peroxide (H_2O_2) determination was carried out following the method of Talaz et al. (2009). Absorbance of solution containing H_2O_2 solution was read against a blank of the phosphate buffer without H_2O_2 at 230 nm. H_2O_2 scavenging activity was expressed, as follows:

$$H_2O_2\ percent\ scavenging = \frac{Abs\ control - abs\ sample}{Abs\ control} \times 100. \quad (4)$$

Brine Shrimp Lethality Assay

The Artemia salina eggs (Brine shrimp) hatched naturally in seawater for forty eight hours. The lethality of the fraction of extract on brine shrimp was conducted as described by McLaughlin (1991). The gradient lethality on the brine shrimp was grouped and categorized, as follows: $LC_{50} \leqslant 100$ µg.mL^{-1} (strongly toxic), LC_{50} = 100–500 µg.mL^{-1} (moderately toxic), LC_{50} = 500-1,000 µg.mL^{-1} (weakly toxic) and $LC_{50} \geqslant$ 1-000 µg.mL^{-1} (nontoxic) (Padmaja et al., 2002)

Cytotoxicity Assessment

Cancer cell lines were obtained from the Centers for Disease Control (CDC), Atlanta, GA, USA and maintained and sub-cultured in the World Health Organization (WHO) Polio Laboratory, Department of Virology, University of Ibadan, Nigeria. HEp-2 (laryngeal cancer cells), Rd (rhabdomyosarcoma), MCF-7 (breast cancer cell), and HeLa (cervical carcinoma) together with normal kidney epithelial cells (vero) from an African green monkey were used. The cytotoxicity assessment of the plant extract on cancer cell lines was carried out following the procedure of Mosmann (1983) where yellow water-soluble tetrazolium was cleaved by succinate-dehydrogenase from living cell into insoluble formazan that was monitored by measuring its absorbance in multiwell spectrophotometer at 492 nm (Mosmann, 1983). The graph of nonlinear regression was plotted between log10 concentration and % cell inhibition and then the IC_{50} was determined using GraphPad Prism software, as follows:

$$\%\ cytotoxicity = \frac{A-B}{A} \times\ 100. \quad (5)$$

The mean of optical density of untreated cells is represented by A , and that of cells treated with plant extract is represented by B. The selective indices (SI) of fractions were derived using noncancerous vero cells where SI greater than one is an indication of cancer selectivity.

$$SI = \frac{CC_{50}\ on\ vero\ cells}{CC_{50}\ on\ cancer\ cells} \times\ 100. \quad (6)$$

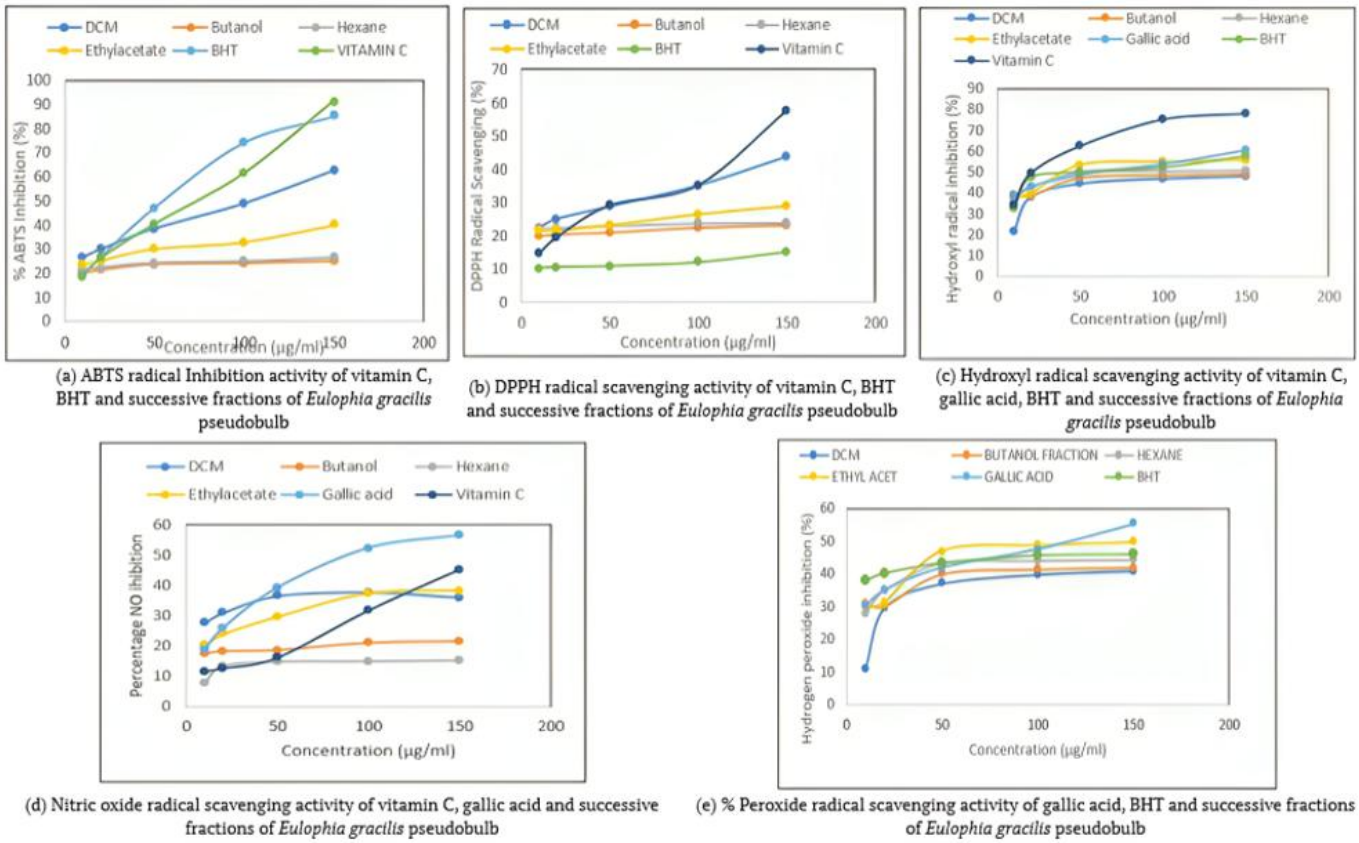
Data Analysis

Data obtained from the cytotoxicity experiments were analyzed by using GraphPad Prism software while data from antioxidant study were analyzed using excel package. The LC_{50} (50% lethality) and CC_{50} (50% cytotoxicity) concentrations were calculated from the dose–response inhibition curve by using nonlinear regression data analysis.

RESULTS

The Total Phenolic Content of Fractions of *Eulophia Gracilis* Pseudobulb

In this present study as presented in **Table 1**, GA was used as standard compound. The value of the total phenolic concentration or content (TPC) of the extract were found to be 55.56 µg GAE/mg for n-hexane, 159.04 µg GAE/mg extract for DCM, 158.65 µg GAE/mg for ethylacetate and 113.74 µg GAE/mg for butanol.



(a) ABTS radical Inhibition activity of vitamin C, BHT and successive fractions of *Eulophia gracilis* pseudobulb

(b) DPPH radical scavenging activity of vitamin C, BHT and successive fractions of *Eulophia gracilis* pseudobulb

(c) Hydroxyl radical scavenging activity of vitamin C, gallic acid, BHT and successive fractions of *Eulophia gracilis* pseudobulb

(d) Nitric oxide radical scavenging activity of vitamin C, gallic acid and successive fractions of *Eulophia gracilis* pseudobulb

(e) % Peroxide radical scavenging activity of gallic acid, BHT and successive fractions of *Eulophia gracilis* pseudobulb

Figure 3. Antioxidant activity of different fractions of *Eulophia gracilis* fractions on ABTS, DPPH, hydroxyl, nitric oxide, and peroxide radicals (the graph are plotted from the mean values of the three replicate values [n = 3]) (Source: Authors' own elaboration)

Table 2. Antioxidant activity of different fractions of *Eulophia gracilis* fractions with their effective concentration 50 (EC_{50})

Fraction	DPPH (EC_{50}) µg/ml	ABTS (EC_{50}) µg/ml	Peroxide inhibition (EC_{50}) µg/ml	Nitric oxide inhibition (EC_{50}) µg/ml	Hydroxyl radical scavenging (EC_{50}) µg/ml
Gallic acid	Not determined	Not determined	114.12	109.06	72.60
BHT	1234.30	65.36	207.03	332.37	81.53
Vitamin C	133.13	70.94	Not determined	173.40	31.61
n-Hexane	1,968.59	849.94	179.27	1,101.41	110.00
DCM	194.03	101.36	179.48	360.31	136.64
Ethylacetate	535.06	246.15	127.28	221.08	78.64
Butanol	1,264.06	939.52	219.15	1,053.23	138.82

Total Flavonoid Content of Different Fractions of *Eulophia Gracilis* Pseudobulb

The result in **Table 1** shows that flavonoid was present in the tested extract and quercetin was used as standard compound and the flavonoid present is expressed in quercetin equivalents. The TFC was found to be 442.78 µg QE/mg for n-hexana 501.94 µg QE/mg for DCM fraction, 271.56 µg QE/mg for ethylacetate and 109.72 µg QE/mg for butanol fraction.

In Vitro Antioxidant Activity

The DPPH radical scavenging, ABTS scavenging, peroxide inhibition, nitric oxide inhibition and hydroxyl radical scavenging potential of the successive extract and the standard controls (GA, BHT, and quercetin) at varied concentrations (10, 20, 50, 100, and 150 µg/ml) are shown in **Figure 3** and computed as effective concentration 50 (EC_{50}) in **Table 2**. The inhibition and scavenging activities are in dose dependent, and all the fractions are compared well to the standard positive controls (GA, BHT, and vitamin C). The DCM fraction is most potent in DPPH and ABTS radical scavenging activities among the extract fractions while ethylacetate fraction has highest inhibitory potential against peroxide radical, nitric oxide and hydroxyl radicals followed by DCM fraction, as shown in **Table 2**.

Table 3. BSL test of successive fractions of *Eulophia gracilis* extract

Fraction	LC_{50} (µg·mL^{-1})
n-Hexane	120.61
Dichoromethane	13.85
Ethylacetate	55.62
n-Butanol	90.68

Brine Shrimp Lethality Test

Table 3 shows BSL test of different fractions of *Eulophia gracilis* extract. The BSL model is a useful screening model in

Table 4. Cytotoxicity effect of successive fractions of *Eulophia gracilis* plant on Rd, HEp-2, MCF-7, HeLa, and vero cell lines

Fraction	CC_{50} (µg.mL−1)					IS (CC_{50} on vero cells/ CC_{50} on cancer cells)			
	RD	HEp-2	MCF-7	HeLa	Vero	RD	HEp-2	MCF-7	HeLa
n-Hexane	26.46	2.42	10.99	4.55	2.27	0.09	0.94	0.21	0.50
Dichoromethane	8.65	0.79	2.84	3.40	9.15	1.06	11.60	3.22	2.70
Ethylacetate	29.86	0.84	1.41	3.17	2.64	0.09	3.15	1.88	0.83
n-Butanol	9.29	1.05	2.95	1.24	5.26	0.57	5.00	1.78	4.23

Note. CC_{50} = 50% cytotoxic concentration in MTT assay & SI = CC_{50} on vero cells/ CC_{50} on cancer cells

preliminary drug design and cytotoxic agent synthesis. Dichoromethane fraction of the extract was most cytotoxic on brine shrimp compared to other fractions. Ethylacetate fraction is slightly more cytotoxic than n-butanol fraction and n-hexane is least cytotoxic among the fractions.

Cytotoxicity Test

Table 4 shows the cytotoxicity effect of different fractions of *Eulophia gracilis* pseudobulbs on Rabdomyosarcoma, HEp-2, MCF-7, HeLa, and vero cell lines. All the solvent fractions of the extract are cytotoxic on selected cell lines. However, DCM fraction is most cytotoxic on RD and HEp-2 cell lines and least toxic to vero which is a normal cell line. Also, ethylacetate fraction is most cytotoxic to MCF-7 and HeLa cell lines. Moreover, DCM fraction seem to be cytoselective for all the selected cell lines used for the cytotoxicity assay. The cytoselectivity is closely followed by n-butanol fraction that is selective for HEp-2, MCF-7, and HeLa cell lines.

DISCUSSION AND CONCLUSIONS

Phenolics are secondary metabolites derived from tyrosine and phenylalanine and occur ubiquitously in plants with various diversities (Naczk & Shahidi, 2004). TPC of the *Eulophia gracilis* was measured exploring Folin-Ciocalteu technique (Okur et al., 2018). Plants with high phenolic content are valuable in the food industry as they reduce the oxidative lipid degradation and enhanced the nutritional value and quality of food (Nardini, 2022). The TPC of fractions of *Eulophia gracilis* extract was evaluated as represented by GA equivalent and the value was found to be 55.56 µg GAE/mg, 159.04 µg GAE/mg, 158.65 µg GAE/mg, and 113.74 µg GAE/mg for n-hexane, DCM, ethylacetate and butanol, respectively. Phenolic molecules display redox potential and can act as antioxidants. The result obtained showed that phenolics is relatively present in good amount in the fractions of extract with highest concentration found in DCM fraction. The attention given to phenolic in scientific research is mainly due to wide-spread in the plant kingdom and being a reliable source of potential natural antioxidants as they are efficient radical scavengers and metal chelators (La Torre et al., 2021). Phenolics displayed radical-scavenging capability because they can donate hydrogen (Naczk & Shahidi, 2004).

Flavonoid is one of the several categories of phenolic compounds which possesses antioxidant activity (Nunes et al., 2012). The mechanism of anticancer activity of flavonoids have been well stated where it inhibits cell proliferation, cell growth, invasion, and stimulation of apoptosis (Çetinkaya et al., 2022). It occurs naturally in plants and imparts positive effects on the health of humans. Studies revealed that flavonoid derivatives exhibited various biological activities such as anti-allergic, antibacterial, anti-inflammatory, antiviral and anticancer activities (Di Carlo et al., 1999; Montoro et al., 2005). They scavenge oxidizing molecules and free radicals that initiated some diseases including cancer (Bravo, 1998). The TFC obtained in this plant are 442.78 ± 19.19 µg QE/mg, 501.94 ± 33.39 µg QE/mg, 271.56 ± 6.74 µg QE/mg and 115.00 ± 5.46 µg QE/mg for successive extraction with n-hexane, DCM, ethylacetate and butanol, respectively. The antioxidant activities of flavonoids in living system have already been reported due to their abilities to scavenge free radical and singlet oxygen and therefore validated that antioxidants and free radical scavengers are present in the extract (Olugbami et al., 2015). Our result however showed that the flavonoid contents of different solvent fractions are higher in quantity when compared to phenolic content in contrast to earlier results obtained by some other researcher (Laloo & Sahu, 2011; Oyedemi et al., 2010).

Determination of ABTS and DPPH radical scavenging activities are commonly used techniques in evaluating the antioxidant that donate proton in plants. The evaluation of DPPH is established on the principle that DPPH has the capacity to be decolorized in the presence of antioxidants, therefore the value of the extent of bleaching corresponds to good scavenging ability (Halliwell & Gutteridge, 2015). The extent of color change is related to the number of antioxidants. A decrease in absorbance of the reacting solution corresponds to bleaching ability and free radical scavenging activity of the tested substance (Krishnaiah et al., 2011). The successive fractions of the extract showed a high DPPH inhibition percentage where DCM fraction was most effective with IC50 = 194.03 µg/mL comparable to ascorbic acid standard of IC50 = 133.13 µg/mL. In ABTS radical scavenging assay, ABTS is oxidized by potassium persulfate to form radical cation of ABTS, which was reduced by antioxidants that can donate hydrogen. This is measured spectrophotometrically at 745 nm. The values of IC50 for ABTS radical scavenging capability of different fractions of *Eulophia gracilis* plant are 849.94, 101.36, 246.16 µg/ml, and 939.52 µg/ml for hexane, DCM, ethylacetate and butanol as compared to the activity obtained from ascorbic acid 75.71 µg/ml and BHT 65.36 µg/ml which are standard antioxidants. These fractions of the plant extract scavenged the ABTS radical in a dose dependent manner. The lower the value of EC_{50}, the higher the antioxidant activity of a sample. The EC_{50} of the DCM fraction in DPPH and ABTS radical scavenging is relatively close to the values of ascorbic acid and BHT which are known antioxidant molecules. The results suggested the presence of phytochemical molecules in the plant extract that can donate hydrogen and therefore scavenge the potential damage of free radical.

H_2O_2 was reported to be present in minute quantities in humans, plants, microorganisms and food (Gülçin et al., 2005). The rapid decomposition of H_2O_2 to water and oxygen may produce hydroxyl radicals (•OH) that is implicated in DNA damage (Sahreen et al., 2011). The result of this study showed that n-hexane, DCM, ethylacetate and butanol fraction of this plant extract efficiently scavenged the H_2O_2 in dose dependent with IC50 of 179.27 µg/ml, 179.47 µg/ml, 127.28 µg/ml, and 219.15 µg/ml, respectively as compared to the standard antioxidant GA of IC50 114.12 µg/ml and BHT of 207.03 µg/ml. Therefore, antioxidant potential of n-hexane, DCM and ethylacetate fractions are higher than that of BHT in scavenging H_2O_2. In addition, H_2O_2 decomposed to hydroxyl radical that initiated damage to cell by its oxidative interaction with fatty acid component of membrane lipid (Khan et al., 2012). Hydroxyl radicals are implicated in pathophysiological processes such as carcinogenesis, mutagenesis and cytotoxicity as they are capable of damaging almost all biological molecules in the living system (Babu et al., 2001). Therefore, the ability of plant extract to scavenge hydroxyl radical is directly proportional to its antioxidant activity (Gülçin et al., 2005). Different fractions of this plant are effective in scavenging hydroxyl radicals in dose dependent manner with IC50 value relatively close to that of standard antioxidant molecule ascorbic acid, BHT and GA, as shown in **Table 2**. The efficient scavenging activity of *Eulophia gracilis* on H_2O_2 and Hydroxyl radical may be linked to phenolic groups that can neutralize H_2O_2 to water by donating electrons to it. This agrees with the finding that phenolic content correlates positively with antioxidant activities (Rodriguez-Bonilla et al., 2017).

Studies have related the antioxidant activity of phytochemicals to their antiproliferative potential where molecule with strong antioxidant capability displayed corresponding potent antiproliferative effect (Ramadan et al., 2019). Phytochemicals such as flavonoids that were isolated from some medicinal plants like *Aloe vera* has been reported to have cytotoxic activity against human breast carcinoma and MCF-7 cell lines (Joby et al., 2014). Therefore, the cytotoxicity potency of plants may be traced to flavonoid content and other phytoconstituents of antioxidant value (Chandu et al., 2012). The BSL model is an important screening model for synthesis of cytotoxic compounds and drug design (Nazir et al., 2013). The DCM fraction of *Eulophia gracilis* extract was most cytotoxic on the brine shrimp followed by ethylacetate fraction, then butanol fraction and lastly by n-hexane fraction. The DCM fraction of *Eulophia gracilis* demonstrated similar cytotoxicity on Rhabdomyosarcoma and HEP-2. cell lines. However, the ethylacetate fraction extracts was more cytotoxic on MCF-7 than other fractions whereas butanol fraction was most cytotoxic on HeLa cell line. The major shortcoming in the development of novel antineoplastic drug is that they lack selectivity on normal noncancerous cells. The inclusion of kidney epithelial cell line (vero) from African green monkey was to determine selectivity. The n-hexane fraction was most toxic to vero cell than all other fractions as presented in **Table 4**. Among the fractions of extract evaluated, DCM fraction appears to be safer on normal cells with IC50 = 9.148 µg/mL.

The safety of the successive fractions of the extract were investigated considering their SI using noncancerous vero lines. The fraction with SI > 1 when cancer cells were compared side by side to normal cells is considered selective for cancer cells in **Table 4**. n-hexane fraction of the extract lacked selectivity (SI = 0.09 for Rd, 0.94 for Hep-2c, 0.21 for MCF-7 and 0.50 for HeLa) on all the four cancer cell lines used in this work. Ethylacetate fraction and n-butanol were also not selectively toxic to RD cell line (SI = 0.09 and 0.57, respectively) while the DCM fraction was selectively toxic to RD cell line with SI of 1.06. Moreover, the three fractions of the plant extracts DCM, ethylacetate and n-butanol fractions are selective for the two human cancer cells (HEp-2 and MCF-7) and may be regarded as fit for chemotherapy of these cancers. Furthermore, only DCM and n-butanol fraction are selective for HeLa cell line and therefore may be considered fit for development of its chemotherapeutic treatment.

Overall, the different successive extracts of *Eulophia gracilis* demonstrated antiproliferative activity and selective cytotoxicity against cancer cells under test with DCM fraction being most active and selective among all. The antiproliferative activities of this plant may be due to the presence of phytoconstituents with characteristic antioxidant properties. Further study is therefore recommended to determine the specific bioactive compounds present in this plant and their mechanisms of cytotoxicity.

Author contributions: OSO: designed the study, carried out benchmark and data collection, and wrote the first draft; **MAG:** provided technical knowledge on methodology; **AJA:** provided cell lines and laboratory equipment; **OAO:** supervised the study and revised the manuscript. All co-authors agree with the results and conclusions.

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Declaration of interest: No conflict of interest is declared by the authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from corresponding author.

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