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Original Research Article

Phytochemical screening, antiproliferative and apoptotic activities of the root bark extract and fractions of *Cola rostrata (Sterculiaceae) K. Schum*. in MCF-7 cell line

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Abstract

Background: Some *Cola* species are used in folklore medicine for the management of cancer related illnesses; thus, the objective of this work was to determine the phytochemical constituents, anti-proliferative and apoptotic activities of crude extract and fractions from the root bark of *Cola rostrata* on MCF-7 cells line.
Methods: The root bark of *Cola rostrate* was collected, identified, cleaned, air dried, pulverized, extracted with methanol and fractionated into petroleum ether, chloroform, ethylacetate and butanol and these fractions were concentrated *in vacuo*. The MCF-7 cells lines were treated with 10 µg/ml of the crude extract and fractions of *Cola rostrata*.
Results: The cells cycle progression showed that the crude extract had an apoptotic activity with 15.96 % of cells undergoing apoptosis as compared to 6.26 % of cells treated with DMSO, showing 9.70 % increase in population of apoptotic cells ($P < 0.001$). Also chloroform fraction treated MCF-7 cells gave 30.30 % proliferation which showed a significant decrease when it was compared with DMSO treated cells of 39.34 %, indicating 9.04 % reduction in proliferative cells ($P < 0.01$).
Conclusion: These results provide evidence that *Cola rostrata* crude extract induces antiproliferative action, while the chloroform fraction induces apoptotic activity in MCF-7 cell line and could be used in the management of MCF-7 cell line ailments.

Keywords: *Cola rostrata*, MCF-7, Flow cytometer, Apoptosis, Antiproliferation

Indexing: Index Copernicus, African Index Medicus

Introduction

Medicinal plants possess active secondary metabolites that enable them to exert their effects and, in the process, giving useful synthetic clues of modern drugs [1], as seen in the discovery of vinblastine and vincristine from Madagascar periwinkle (*Catharanthus roseus*). This may have paved way for the use of plant materials in the management of cancer [2].
In Nigeria, it is estimated that 75 % of the people living in rural areas, 40 % in urban areas depend on herbs for their healthcare need due to high cost of orthodox medicine, unbearable side effects and most importantly the strong believe in traditional medicine especially among the rural populace [3].
Cancer is an uncontrolled growth and spread of malignant cells that may affect most tissues of the body. Breast cancer is the most common malignancy and a leading cause of death in females (14 %). Recently it was discovered to arise from a number of molecularly-distinct tumors arising from the epithelial cells of the breast [4].
Apoptosis is the process of programmed cell death, characterized by cell shrinkage, chromatin condensation, caspases activation and DNA fragmentation [5]. While proliferation is an essential

portion of cancer progression and development. It is observed by change in appearance and action of cell cycle linked proteins [6].

Cola is the largest genera in the family sterculiaceae; it is native to tropical rain-forest of Africa and comprises about 125 species of trees [7]. It is useful in folklore medicine for the management of hypertension, treatment of migraine, dysentery and parasitic diseases it is also used as an antiemetic, a mild stimulant, an appetite supressant, a diuretic, laxative and to stop bleeding, and to treat cancer associated illnesses [8,9,10,11]. *Cola rostrata* is a perennial tree found in lowland rain forest of tropical Africa; in southern Cameroun, Gabon and south-eastern Nigeria and its fruits are edible with a sweet taste. Different species of *Cola* have been reported to be used in the management of cancer related ailment [12]. Literature search show that there is paucity of information with regard to the antiproliferative and apoptotic activities of *Cola rostrata*, thus this present study was designed to evaluate the antiproliferative and apoptotic activities of the root bark of *Cola rostrata* in MCF-7 cells line.

Materials and Methods

Collection and preparation of root bark of *Cola rostrate*

The root bark of *C. rostrata* was collected on the 30th June, 2011 from Ohanozara, Ebonyi State, Nigeria. The plant was identified and authenticated by Ugbogu O. A. and Shansanya of the Forest Research Instituted, Ibadan, Nigeria (FRIN) and voucher specimen number (FHI 109430) was deposited. The root bark was carefully washed to remove sand and stones from the sample, it was then air dried away from direct sunlight for 4 weeks, this was then pulverized using electrical milling machine, before placing it in an airtight container.

Extraction and fractionation of powdered root bark of *Cola rostrata*

Powdered root bark (2.9 kg) of *C. rostrata* was macerated with 17 L of methanol for 72 hours. The filtrate was evaporated using rotary evaporator at 40^0C under reduced pressure and the weight was noted. The concentrated extract was stored in a refrigerator at 4^0C until used.

The crude extract (125 g) was dissolved with 60 ml of methanol and then 240 ml of water was added. These were placed in a separating funnel and fractionated into 2x50 ml of petroleum ether. The petroleum ether fraction was collected and this procedure was repeated and the petroleum ether fractions was put together after the solvent became clear. The same procedure was repeated for chloroform, ethylacetate and butanol respectively. The various fractions were concentrated *in vacuo* and their weight was noted.

Phytochemical screening: The phytochemical screening was carried out by using already established method [13,14].

Cell culture: All cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA) and maintained at 37ºC and in 5% CO_2 atmosphere in a monolayer. Confluent cells were passaged by treating them with 0.05% trypsin-0.02% EDTA.

Treatment with plant extract and fractions: For all experiments 0.5 x 10^6 cells were seeded in a 6-well plate in regular culture medium for 24 hours. Subsequently, cell lines were washed with phosphate buffer saline (PBS) and adapted to phenol-red-free Dulbecco's modified Eagle's medium for 48 hours to avoid unspecific stimulation of endogenous hormones in the serum. MCF-7 cell lines were treated with 10 µg/ml of the crude extract and fractions and 0.1% DMSO (control) for 48 hours.

Flow cytometric measurement of cell proliferation: The extent of cell cycle progression and apoptosis was estimated by flow cytometric analysis [15]. For statistical evaluation, the S-phase and G2/M-phase cells were defined as proliferative cells.

Statistical analysis: Every experiment was replicated three times; data sets were expressed as mean ± standard deviations (SD). Statistical significance was determined by unpaired t-test (***P < 0.001, **P < 0.01, *P < 0.1).

Results

The weight of the methanolic extract obtained from the maceration of 2900g of *C. rostrata* powdered root bark with 17 L of methanol was 132.0650g (Table 1). This was then partitioned into petroleum ether, chloroform, ethylacetate and butanol (increasing polarity). The weights of the fractions were obtained as 6.1875g, 1.5500g, 0.8000g and 0.2625g respectively, after evaporating in vacuo at 60 ºC.

Table 2 shows the result of the phytochemical screening of the powdered root bark of *C. rostrata*. The presence of saponins, tannins, reducing sugar, triterpinoids, alkaloids and flavonoids. The percentage proliferative MCF-7 cells after treatment with *C. rostrata* crude extract and DMSO was (33.25 ± 1.50 %) and (31.88 ± 0.61 %) respectively (Figure 1Illustrating a (1.37±0.89 %) increase in MCF-7 cells.

Figure 2 illustrates the percentage apoptosis of human breast adenocarcinoma cell line (MCF-7) after treatment with the crude extract of *C. rostrata* and DMSO. The crude extract caused a 9.71% increase in apoptosis against MCF-7 cell lines compared to DMSO (15.97 ± 3.75 % for crude extract and 6.26 ± 0.88 % for DMSO), ($p< .0.001$).

The percentage number of MCF-7 cells in the G1-phase after treatment reduced by 1.36%. However, there was a 0.91% and 0.46% increase in the percentage number of cells in the S and G2-phases respectively. $p > 0.05$ (Figure 3).

Table 1: weight of the extract and fractions of *Cola rostrate*

Extract/Fractions	Weight (g)
Methanol	132.0650
Petroleum ether	6.1875
Chloroform	1.5500
Ethylacetate	0.8000
Butanol	0.2625

Table 2: Phytochemical constituents of the root bark of *Cola rostrate*

Phytochemicals	Inference
Flavonoids	+
Alkaloids	+
Saponins	+
Triterpenoids	+
Tannins	+
Reducing sugars	+

Present (+); Absent (-)

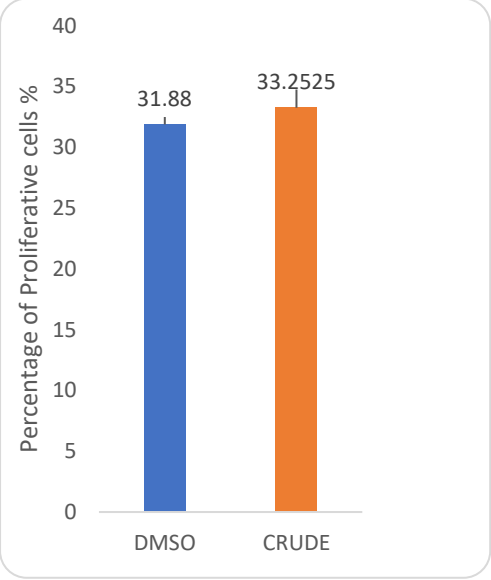

Figure 1: Percentage of proliferation of human breast adenocarcinoma cell line (MCF-7) after treatment with the crude extract of *Cola rostrata* and DMSO.

Following the fractionation of the methanolic extract, the different fractions were evaluated against MCF-7 cell line, it was observed that the percentage proliferation of the petroleum ether (PE), chloroform (CH), ethylacetate (EA) and butanol (BU) fractions were (34.58 ± 0.23 %), (30.30 ± 0.73 %), (38.58 ± 0.35 %) and (37.60 ± 0.60 %) respectively (Figure 4). These showed (4.76 ± 1.34 %), (9.05 ± 0.84 %), (0.76 ± 1.22 %) and (1.74 ± 0.97 %) reduction in the proliferative cells. When these were compared against DMSO (39.34 ± 1.57 %), only CH fraction showed significant different of P<0.001.

The extent of apoptosis in MCF-7 cells was evaluated and it was observed that EA fraction showed cell apoptosis of 0.26 ± 0.13 %, PE fraction (0.12 ± 0.06 %), BU fraction (0.10 ± 0.05 %) and CH fraction (0.07 ± 0.05 %) (Figure 5). Showing the extent of apoptosis of by each fraction to be (0.56 ± 0.18 %) EA (0.72 ± 0.26 %) BU and (0.75 ± 0.26 %) CH, (0.70 ± 0.25 %) CH respectively,

The number of cells in the G1-phase post treatment showed (65.42 ± 0.23 %) PE, (69.72 ± 0.72 %) CH, (61.42 ± 0.35 %) ET and (62.40 ± 0.60 %) BU. The cell population in the S-phase was observed to be (20.11 ± 0.59 %) CH, while ET, PE and BU had (13.23 ± 0.73 %), (11.82 ± 0.17 %), and (10.64 ± 0.37 %) respectively. The population of cells in G_2-phase showed was observed to be (10.18 ± 0.14 %) CH, (22.77 ± 0.39 %) PE, (25.35 ± 0.89 %) ET and (26.96 ± 0.27 %) BU. It was observed that the number of cells in the G1-phase increase by (5.1 ± 1.33 %) PE, (9.40 ± 0.84 %) CH, (1.10 ± 1.21 %) ET and (2.08 ± 0.94 %) BU. S-phase showed an increase also by (3.77 ± 0.11%) PE, (12.05 ± 0.53 %) CH, (5.17 ± 0.67) ET and (2.58 ± 0.31 %) BU. While G2-phase of the cell cycle showed a decrease in the number of cells by (8.52 ± 37 %) PE, (21.11 ± 0.62 %) CH, (5.94 ± 0.13 %) ET and (4.33 ± 0.49 %) BU. These results showed that there was significant increase in the number of MCF-7 cells line by 9.40 % and 12.07 % due to the CH fraction ($p < 0.01$) in both the G1 and S-phases.

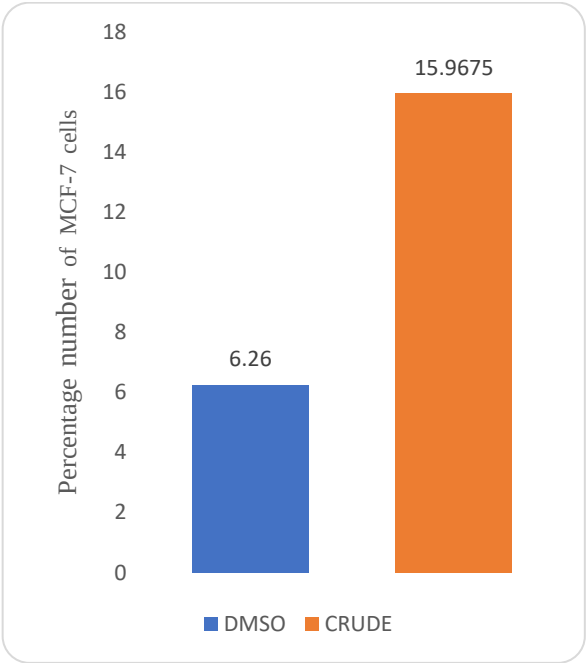

Figure 2: Percentage of apoptosis of human breast adenocarcinoma cell line (MCF-7) after treatment with the crude extract of *Cola rostrata* and DMSO.

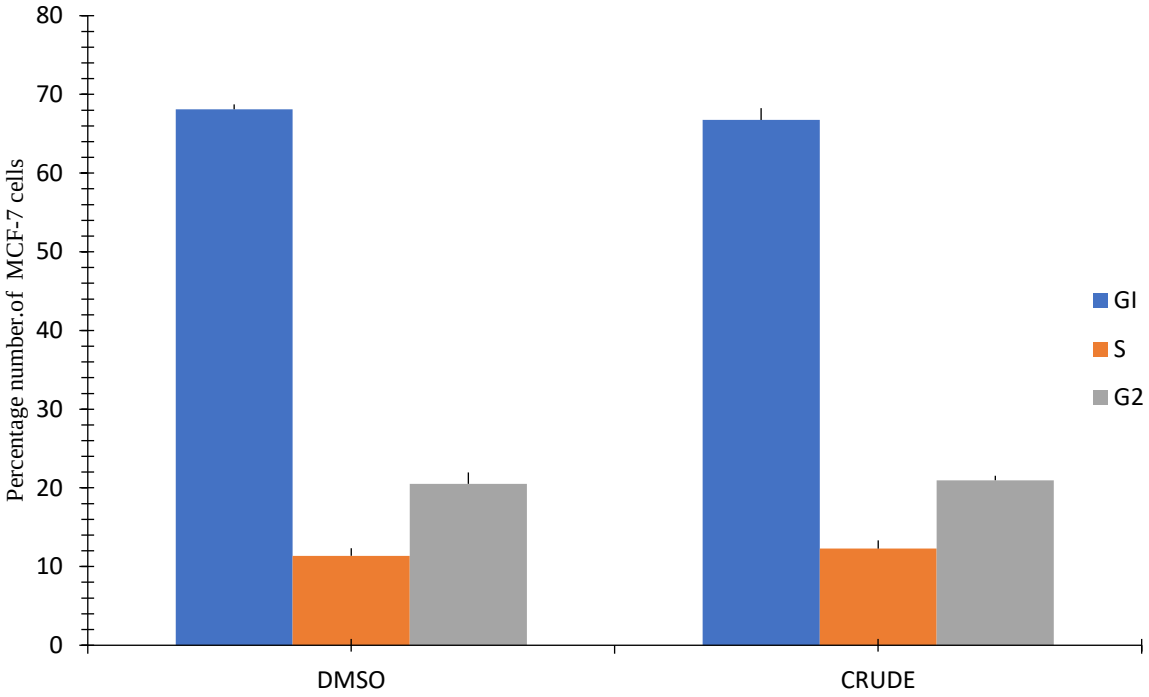

Figure 3: Percentage number of human breast adenocarcinoma cell line (MCF-7) in the different cell cycle phases (G1, S, G2) after treatment with the crude extract of *Cola rostrata* and DMSO.

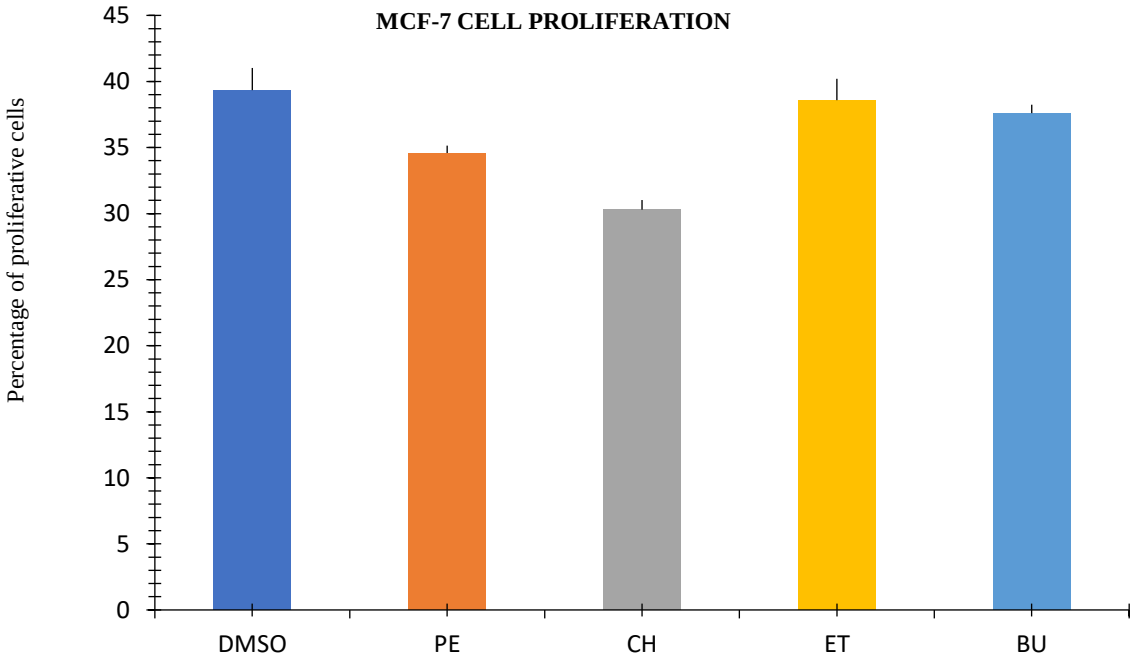

Figure 4: Percentage proliferation of human breast adenocarcinoma cell line (MCF-7) after treatment with the fractions of *Cola rostrata* and DMSO.

PE = Petroleum ether fraction, CH = Chloroform fraction, ET = Ethylacetate fraction, BU = Butanol fraction, DMSO = Dimethylsulphoxide

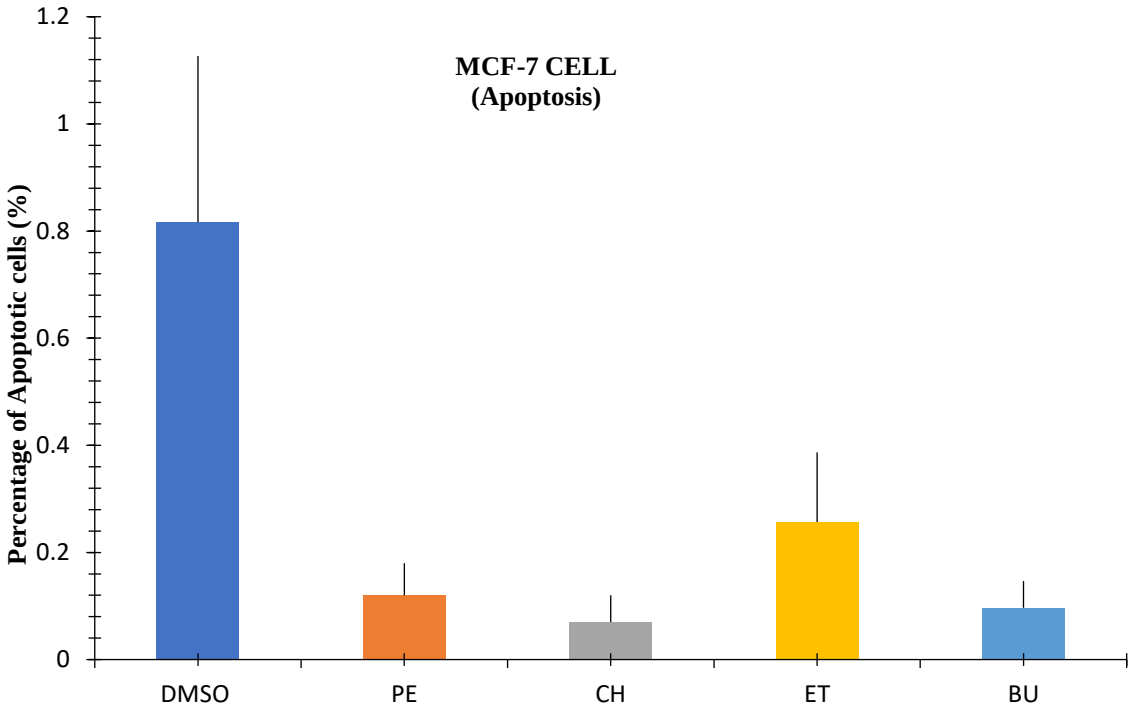

Figure 5: Percentage of apoptosis of human breast adenocarcinoma cell line (MCF-7) after treatment with the fractions of *C. rostrata* and DMSO.

PE = Petroleum ether fraction, CH = Chloroform fraction, ET = Ethylacetate fraction, BU = Butanol fraction, DMSO = Dimethylsulphoxide

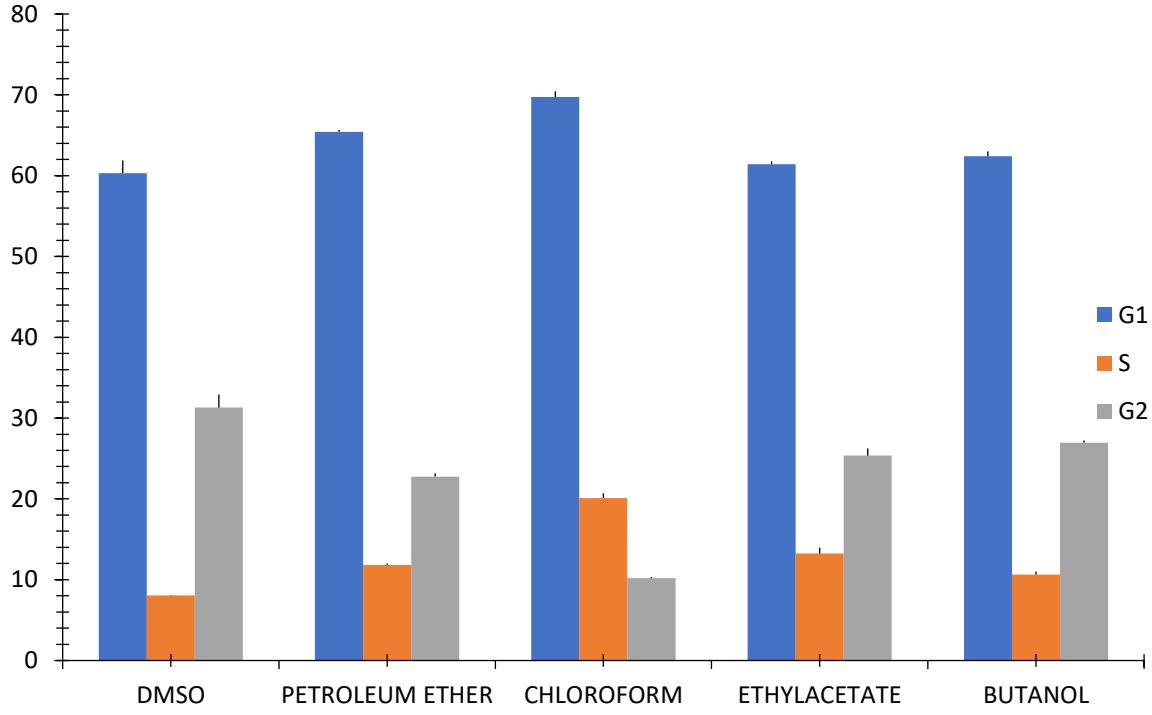

Figure 6: Percentage of number of human breast adenocarcinoma cell line (MCF-7) in the different cell cycle phases (G1, S, G2) after treatment with the fractions of *Cola rostrata* and DMSO.

Discussion

Cancer is managed by surgery, chemotherapy and radiotherapy but due to the side effects associated with these therapies [11], the need for other means of treatment has brought to light the use of herbal preparations. Herbal preparations are believed to be safe, cheap and readily available [16]. The contents of herbs vary in plants and their application ethno-medicinally can be different. Cola species have a rich source of phytochemicals which are responsible for their therapeutic effect as folklore medicines [17]. Their use in the management of cancer related ailment could be ascribed to these phytochemicals which have been reported to alter the growth or development of cancerous cells in human adenocarcinoma cells [18]. However, as far as we know there has been no report on the anti-proliferative and apoptotic activities of *C. rostrata* crude extract and fractions.

Anti-proliferative and apoptotic activities of the crude extract of *C. rostrata* was determined using MCF-7 cell lines in a flow cytometer. Flow cytometry gives the percentage number of cells in each phase of the cell cycle *in vitro* after the extract or fractions may have been administered (Dose = 10 µg/ml), by taking advantage of the cell membrane permeability to dyes used in the assay. A slight increased proliferation of MCF-7 cell line was observed after treatment with the crude extract of *C. rostrata* when compared with cells treated with DMSO used as solvent (Figure 1), while the percentage number of cells undergoing apoptosis increased significantly (9.71 %) (Figure 2). The induction of apoptosis may be due to the decreased percentage number of cells in the G_1 phase with concomitant increase in S and G_2 phases, suggesting a G_1 phase arrest, thus slowing entry of these cells into the S-phase. This is a regulatory process by G_1 checkpoint, preventing the progression of cells with damaged DNA to the next stage of cell cycle. Thus, *C. rostrata* crude extract may have triggered a checkpoint that causes arrest in G_1 phase of the cell cycle.

The fractions were observed to have reduced percentage number of cells in the proliferative stage (figure 4), this was observed more with the chloroform fraction (CH), followed by the petroleum ether fraction (PE). Further evaluation showed that the CH fraction produced the lowest percentage number of apoptotic cells, followed by the butanol fraction (BU). The anti-proliferative activity exhibited by the fractions could be due to their increased number of cells in the G1-phase or increased cells in G2-phase of the cell cycle (figure 6). The altering of the percentage of cells in G1 and G2 phase of the cell cycle may have activated a checkpoint which may be responsible for correcting the damage of the MCF-7 cells due to the administration of the fractions of *C. rostrata*.

The anti-proliferative and apoptotic effects observed in MCF-7 cell lines, may be attributed to the presence of flavonoids, alkaloids, tannins and triterpenoids, that may be present in the crude (methanol) and non/semi polar fractions (CH and PE) [19].

Conclusion

These results provide evidence that *Cola rostrata* root is rich in phytochemicals, the methanol extract possess apoptotic activity on MCF-7 cell line, while the chloroform fraction exerted the highest anti-proliferative activity and could be used in the management of MCF-7 cell line related ailments.

Acknowledgement

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Conflict of Interest

No conflict of interest is associated with this work

Contribution of Authors

The study was conceived and designed by S.A. Adelusi, A. Falodun and E. E. Odion, data was collected by N.L. Engel, Analysis of the data was done by E.E. Odion and work was proof read by E.E. Odion.

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