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Phytochemicals and anticancer activity of methanol extract of *Trigonella foenum-greacum* seed on breast cancer cell lines

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ABSTRACT

This study was aimed to examine the anticancer potential of *Trigonella foenum-greacum* seed methanol extract by analyzing its phytochemicals and its breast cancer cells effects. The anti-cancer efficacy of the extract was tested on fibroblast cells and MCF-7 through various cellular assays, including apoptosis, mitochondrial membrane potential, reactive oxygen species, cell cycle distribution, and cell cytotoxicity assay were all investigated. By using GC-MS analysis, fifty-five bioactive compounds were identified in the extract. When the cancer cell lines were treated with the extract, the typical apoptotic morphology was observed, this resulted in a dose-dependent reduction of cancer cell migration and adhesion. Flow cytometry studies revealed that the extract significantly induced apoptosis by triggering a change from G_2/M to polyploidy (>G) at greater doses. Furthermore, the extract increased mitochondrial depolarization and ROS levels. This study demonstrates the extract's anti-cancer properties and highlights the need for further analysis to understand how *Trigonella foenum-greacum* seed extract induces program cell death through multiple signaling pathways at varying concentrations.

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1. Introduction

Breast cancer is a prevalent form of cancer worldwide and ranks as the third most common cancer, causing approximately 48,000 female deaths each year, with over 50% of cases occurring in developed countries (Łukasiewicz et al., 2021; Turner et al., 2020). It is also one of the most frequent cancer kinds in the Middle East and is a significant threat to women worldwide (Safiri et al., 2022; Salem and Daher-Nashif, 2020). In 2012, 1.57 million breast cancer incidences were officially recorded, making it one of the most concerning diseases affecting women. Currently, breast cancer is considered the fatal illness among women (Azubuike et al., 2018). As reported in Nigeria, breast cancer incidence is presently a total of 54.3 women in 100,000 (Olasehinde et al., 2021). One of the most often used protocols in the treatment of breast cancer is chemotherapy (Al-Timimi, 2019). This protocol's use is frequently accompanied by unfavorable side effects, including as illness, bone marrow suppression, and the emergence of multidrug resistance (MDR) (Al-Timimi, 2019). Therefore, identifying distinctive mixtures in plants may offer a therapy option for malignant growth.

In Nigeria and globally, the anticancer effects of the plant extract have recently attracted a lot of attention. It is becoming more commonplace to recommend these phytochemicals as drugs (Bhalodia et al., 2011). The world population today (about 33%) relies on medicinal plants and plant extracts to meet their basic health needs. The world health organization also reported that 80% of people around the world are habituated to using manufactured drugs (Kumar and Reddy, 2012). Because of the beneficial uses of these plants to treat diseases as well as their extensive medical and economic qualities, the therapeutic effects studies by researchers of plants has recently grown. Some research has revealed that *Trigonella foenum-greacum* seed has healing effects.

Trigonella foenum-greacum, a member of the plant family Leguminosae that is often called fenugreek in English and "helba" in Arabic, is an annual plant cultivated in Asia and Mediterranean countries (Shahrajabian et al., 2021). It includes metabolites with antibacterial characteristics such as tannins, flavonoids, alkaloid terpenoids, and glycosides (Khorshidian et al., 2016). The dry seed aqueous extract

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proved cytotoxic to various cancer cell lines, including B-cell lymphoma, thyroid papillary carcinoma and T-cell lymphoma (El Bairi et al., 2017).

According to various animal model studies, the seed of *Trigonella foenum-greacum* have demonstrated to reduce blood glucose and reinstate some of the functions of crucial enzymes involved in the metabolism carbohydrate and lipid (Turner et al., 2020). Despite the fact that the specific components responsible for and technique by which *Trigonella foenum-greacum* achieves the outcomes are entirely unclear, investigations have discovered the occurrence of steroid saponins in *Trigonella foenum-greacum* seeds (Arivalagan et al., 2013). Saponin compounds, diosgenin, alkaloids, and trigonelline have been shown *in vitro* to limit intestinal glucose absorption (Shehadeh et al., 2021). Furthermore, 4-hydroxyisoleucine, a modified amino acid produced from the seeds of *Trigonella foenum-greacum*, has been demonstrated in animal models of type 2 diabetes mellitus to have an insulinotropic action *in vitro,* increase insulin secretion *in vivo*, and improve glucose tolerance (Alam et al., 2022).

Many studies conducted to evaluate the anticancer properties of *Trigonella foenum-greacum* seeds was extracted either by aqueous (Abas and Naguib, 2019; Almalki and Naguib, 2022; Alsemari et al., 2014; Salam et al., 2023) or ethanol (Al-Timimi, 2019). Also, many of the studies were carried out in the Arab world, i.e., Saudi Arabia (Al-Timimi, 2019; Alsemari et al., 2014) and Egypt (Abas and Naguib, 2019; Salam et al., 2023) but no studies has been carried out with methanol extract and using *Trigonella foenum-greacum* seeds from West Africa particularly Nigeria.

The bioactive compounds found in dietary and herbal plants recently are employed as anticancer medicines. Hence, this study was to evaluate the phyto-chemical composition and the anticancer potential of *Trigonella foenum-greacum* seeds' methanol extract (METFS) against breast cancer cells and fibroblast cells.

2. Materials and methods

2.1. Chemicals and reagents

Gibco Dulbecco's Eagle's medium, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Fetal bovine serum were products of Sigma-Aldrich Inc. St. Louis, MO, USA, Waltham, MA, Thermo Fisher Scientific, USA. MCF-7 cancer cells were selected because they maintain several characteristics similar to mammary epithelium and fibroblast cells served as control. Tissue Culture Laboratory of the Virology Department of College of Medicine, University College Hospital, (UCH) Ibadan, Nigeria was the source of both cells.

2.2. Plant material, authentication, and extraction

In March 2021, *Trigonella foenum-greacum* was procured from Oba's market in Ilorin, Kwara State, Nigeria. The plant was identified and voucher number (UILH/001/1419/2021) was provided by Mr. Bolu Ajayi of the Herbarium Services, Plant Biology Department, University of Ilorin, Ilorin, Nigeria. After the air-dried of the seeds at 37 ° C, they were ground into a fine powder. Hundred (100 g) of the fine powder was macerated in 2 L of 96% methanol for a period of twenty-four hours and the filtrate was attained by Whatman No. 1 filter paper, while the residue was dried to powder with total elimination of the methanol. The yield of the extract in percentage was determined by a formula:

$$\%\ \text{Yield} = \frac{\text{Mass of Extract (g)}}{\text{Mass of Sample (g)}} \times 100$$

2.3. Gas chromatography-mass spectrometry (GC-MS) analysis of the extract

The Gas chromatography-mass spectrometry analysis was performed at the Shimadzu Training center (STC) Lagos, Nigeria. Agilent7890/5975B-GC/MSD as the analytical equipment from Palo Alto, CA, USA, was used with the capillary column of stocked with a HP-5 MS and HP-5975B mass selective detector. The response followed the procedure developed by Sun et al. (2015). At a 1:10 ratio, the sample was mixed with ether and one microliter of the mixed sample was manually inserted with 40:1 ratio. The ionization of the electron for Gas chromatography-mass spectrometry detection was done at 70 eV. The temperature of the oven was initially set to 50 °C for three minutes and gradually raised to 250 °C at a 3 °C/min rate and held at 250 °C for four minutes. The MS and the injector transfer line temperatures were set at 250 and 220 °C respectively and helium served as the carrier gas at 1 mL/min flow rate. By comparing the mass spectra and the retention time to values in the NIST98 GC–MS library and in relevant texts, the components were identified.

2.4. Preliminary phytochemical screening and radical scavenging activity

Standard procedures were used to conduct preliminary phytochemical screening of the methanol extracts (Harbone, 1984; Daniyan et al., 2011a, 2011b). Alkaloids, tannins, steroids, flavonoids, carbohydrates, glycosides, anthraquinone, reducing sugar, and phenol were among the compounds tested in the extract.

By the procedure of Slinkard and Singleton (Hatami et al., 2014), the total phenolic content (TPC) was assay utilizing the Folin-Ciocalteu reagent. Briefly, using 50% ethanol, ten percent solution was produced from the stock (30 mg/ml). The solution was then mixed with 200 μl of the Folin-Ciocalteu reagent and 2 ml of de-ionized water and incubated at room temperature for 3 min. After 1 h of incubation at room temperature. The same process was used to create a negative control sample. The absorbance of the ensuing blue-hue was measured at 765 nm and represented in mg gallic acid equivalents (GAE) per g dry weight of plant material. For this, an equation developed from the gallic acid calibration curve was utilized. The samples were examined three times.

Colorimetric technique with aluminum chloride was used to measure the total flavonoid concentration in the extracts (Zhishen et al., 1999). Methanol served as solvent to prepare 600 μg/ml concentration of the extract. The extracts (500 μl) were combined individually with 1.5 ml of methanol, 0.1 ml of 1 M potassium acetate solution, 2.8 ml of distilled water and 0.1 ml of 10% (w/v) $AlCl_3$. The absorbance at 415 nm was determined after thirty minutes at room temperature. The quantification of the total flavonoid content was done as mg of quercetin equivalents (QE)/g of dry extract using a standard calibration with quercetin.

The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical as scavenged by the extract capability was evaluated to determine their antioxidant activity (Naji et al., 2020). At different concentrations, the extract was prepared in methanol (0.15–1.5 mg/ml). In a test tube, a DPPH methanol solution (3.8 ml, 60 g/ml) was applied to the extract of the plant (200 l, 30 mg/ml), as a control was assayed concurrently, methanol was employed as a blank sample. The tubes were spun and at room temperature, the contents were maintained in the dark for 30 min. The scavenging power of the plant extract was determined using the following equation as the absorbance was measured at 517 nm:

$$\text{Scavenging activity of DPPH}\ (\%) = \frac{(Abs\ Blank - Abs\ Sample)}{(Abs\ Blank)} \times 100$$

where;

Abs blank = control absorbance

Abs sample = sample absorbance.

The ability of the plant extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl radicals was represented as IC_{50} (mg/mL), which is the amount of concentration necessary to scavenge fifty percent of the 2,2-diphenyl-1-picrylhydrazyl radicals. The set-up was replicated.

Graphical investigation was used to determine the IC_{50} value (μg/ml). A value that represents the concentration when 50% of the stable DPPH· radicals are scavenged. The total antioxidant activity was calculated in respects of ascorbic acid equivalent per gram of dry extract. The experiment was carried out in triplicate.

2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay was used to investigate the radical scavenging ability of *Trigonella foenum-greacum* seed extract (Souza et al., 2019). Stock solutions of 2.45 mM potassium persulfate ($K_2S_2O_8$) and 7 mM ABTS were prepared separately using distilled water. By equal addition of the two stock solutions, incubation was done for 16 h at 25 °C in the dark. One milliliter of ABTS solution was mixed in sixty milliliters of methanol to create a new working solution that consistently read 1.1 ± 0.02 at a wavelength of 734 nm. Next, 150 μg of *Trigonella foenum-greacum* seed extract was combined with the absorbance, 2.85 mL of ABTS solution and after incubating in the dark for 2 h, was read at 734 nm. The blank was methanol, and the scavenging activity of the seed extract was estimated by the equation:

$$\text{Scavenging activity ABTS radical } (\%) = \frac{(Abs\ control - Abs\ Sample)}{(Abs\ control)} \times 100$$

According to Naji et al. (2020), the ferric ion reducing antioxidant potential (FRAP) assay is a method for evaluating a substance's antioxidant activity depending on its capacity to reduce a ferric ion-2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) complex. This reaction resulted in the formation of a dark blue color due to the bond between Fe^{2+} and the ligand. The absorbance of this color is measured as the quantity of reduced iron, which is relative to the antioxidant's sample concentration.

2.5. Cytotoxicity assay

Fibroblast cells and the plant extracts cytotoxicity on MCF-7 was assay by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay (Jones et al., 2020). In brief, cell lines were placed in a 96 well plate with a concentration of 1×10^4 cells in a well and these were treated with different plant extract concentrations for twenty-four hours at 37 °C in a CO_2 incubator. At the end of the treatments, phosphate buffer saline was used to wash the cells and incubated with MTT solution in the medium for 4 h. To dissolve formazan crystals produced in live cells, Dimethylsulfoxide was used, and the intensity of the resulting color was determined by an ELISA reader at 570 nm after overnight incubation in the dark. The formula below was used to compute the cell viability percentage.

$$\text{Cell viability } (\%) = \frac{\text{Abs of treated cells}}{Abs\ of\ control\ cells} \times 100\ \%$$

2.6. Cell cycle and apoptosis assay

DNA content was measured using propidium iodide (PI) to assess cell cycle distribution (Alabi et al., 2020). Cells (3×10^5) were plated in 6 cm petri-dishes containing 3 ml media and incubated for 24 h. After treatment with the extract at designated doses, the incubation of the cells was repeated for 48 h. After which all the cells collected and fixed overnight in seventy percent ice cold ethanol in buffer. Sample buffer was used to wash the dishes and cells were suspended in 50 μg/ml PI and 100 μg/ml in RNAse A sample buffer and incubated for thirty minutes at 37 °C.

At a density of 2×10^4 cells/well in a 96 well plate, the cells were seeded and allowed to attach for twenty-four hours. Subsequently, the cells were cultured for a further twenty-four hours at the changed media to media containing the extract at various concentrations (100 to 500 μg/ml). Cell Death Detection ELISA PLUS kit was employed to evaluate apoptosis, which detects the release of histones from fragmented DNA in apoptotic cells (Alabi et al., 2020). The assay employs a concept of sandwich enzyme immunoassay, with monoclonal antibodies from mouse specific to histones and DNA, allowing the exact detection of mono- and oligo-nucleosomes in cell lysates. In 200 μl of lysis buffer at room temperature for thirty minutes, the cells were lysed and after centrifuging the lysate for 10 min, 200 μl of supernatant was recovered. At room temperature, 20 μl of this was treated by anti-histone biotin and anti-DNA peroxidase for two hours. With incubated buffer, the wells were washed three times, after which 100 μl of 2,2-azinodi (3-ethylbenzthiazoline-sulphuric acid) as substrate solution was pipetted into respectively well and incubated at 25 °C for 20 min. The absorbance was read thrice by the use of ELISA reader (Jenway Spectrophotometer, UK)

2.7. Mitochondrial membrane potential

By carefully following the manual instructions, the changes in the mitochondrial membrane potential with flow cytometry was determined using TMRE-Mitochondrial Membrane Potential assay kit (Abcam, Cambridge, USA). Cells (2.5×10^5) were pipetted into each well of a 6-well plate and incubated for twenty-four hours. Each well was treated with designated doses followed by 48 h incubation. Subsequently, the cells were extracted, washed, and treated with 200 nM TMRE at 37 °C for twenty minutes. The acquired data was analyzed through FlowJo software after being run on MACSQuant Analyzer 10.

2.8. Determination of reactive oxygen species (ROS) generation

The DCFDA/H_2DCFDA-Cellular reactive oxygen species assay kit was employed to determine the level of RO by flow cytometry. For 24 h, cells-(2.5×10^5) were planted in 6-well plates, treated with the extract at 0, 40, 80, 100, and 150 μg/ml, followed by 48 h incubation. Twice, the cells were washed after harvesting with PBS followed by incubation with DCFDA (20 μM) at 37 °C for thirty minutes. The samples images were obtained with MACSQuant Analyzer 10 and FlowJo software was used for the analysis.

2.9. Statistical data analysis

Obtained data in this study were presented as SEM from the three replicates using ANOVA with Bonferroni's analysis by Graph-Pad Prism, v. 9.5.

3. Results

3.1. Phytochemical analysis of the extract

Table 1 displays the result of the initial screening for phytochemicals, which showed the presence of prominent categories including tannins, alkaloids, saponins, and others.

3.2. GC-MS results of the extract

Table 2 displays many components with diverse retention times that were contained in the extract as indicated by gas chromatography-mass spectrometry results. This is illustrated by the spectrum in Fig. 1. The analysis identified a range of 55 bioactive compounds in the *Trigonella foenum-graecum* seed extract, highlighting the significance of these seeds in traditional medicine.

Table 1
Phytochemical characteristics of the methanolic extract of *Trigonella foenum-greacum* seeds.

Test	Name of the test	Methanolic maceration extract
Saponins	Frothing test	+
Alkaloids	Wagner's test and Hager's, Meyer's	+
Flavonoids	Shinoda test	–
Steroids	Salkowski test	+
Glycosides	Nitroprusside test	+
Carbohydrates	Molisch's test	+
Tannins	$FeCl_3$ test	+
Anthraquinone	Anthraquinone test	+
Reducing sugar	Reducing sugar test	–
Phenol	Phenol test	+

'**+**' shows the presence of the phytochemical in the extract, '**-**' shows the absence of the phytochemical in the extract.

3.3. Preliminary phytochemical screening and radical scavenging activity

The *Trigonella foenum-greacum* seed extract was evaluated for the presence of polyphenols as TPC and TFC. The results show that TPC and TFC in the extract were 128.3 and 14.93 μg/g respectively. The antioxidant activity of the *Trigonella foenum-greacum* seed extract was found to be dependent on the dosage. The substrate concentration (SC_{50}) value for DPPH and ABTS was 11 and 19 μg/ml (Table 3). Lower value of SC_{50} shows elevated activity of antioxidant. Proton free radicals are found in DPPH and ABTS molecules that were considerably decreased upon exposure to proton radical scavengers. The antioxidant activity of the *Trigonella foenum-greacum* seed extract was measured using the FRAP test by assessing power reduction, which indicates electron donation. This assay measured that the reducing power of the *Trigonella foenum-greacum* seed extract was

Table 2
Gas chromatography-mass spectrometry of the methanolic extract of *Trigonella foenum-greacum* seeds.

Peak	Real time	Area	Height	Height %	A/H	Compound Name
1	5.38	4,999,177	235,444	0.97	2.12	Methoxyacetic acid
2	6.35	8,185,741	873,212	0.36	9.37	2,5-Dimethyl-4-hydroxy3(2H) furanone
3	6.63	4,825,415	795,681	3.28	6.06	2-Propen-1-amine
4	6.77	3,587,944	841,363	0.35	4.26	Maltol
5	6.93	1,200,665	423,240	1.74	2.84	Cyanic acid
6	7.61	4,471,841	173,286	0.71	2.58	Methylphosphonic acid
7	8.03	2,141,924	242,172	1.00	8.84	Dihydrobenzofuran
8	8.50	1,842,495	418,948	0.17	4.40	1,1-Cyclobutanedicarboxylic acid
9	9.22	6,593,954	259,845	1.07	2.54	Eugenol
10	9.39	1,551,153	482,844	0.20	3.21	Hydrazine
11	9.83	2,925,476	118,505	0.49	2.47	Pyrazol-3-one
12	10.17	2,490,542	458,951	0.19	5.43	4-Penten-2-one
13	10.28	3,950,410	800,724	0.33	4.93	Bicyclo[2.2.1]heptan-2-one
14	10.52	4,104,417	223,086	0.92	1.84	1-Pyridineacetic acid,
15	10.57	7,325,309	197,506	0.81	3.71	Tricyclo[4.4.0.0(3,9)]decane
16	10.83	9,407,355	228,199	9.40	4.12	3-Cyclohexene-1-ethanol
17	11.05	2,234,022	235,155	0.97	9.50	9-Oxabicyclo[4.2.1]nonan-2-ol
18	11.20	7,024,550	655,204	2.70	10.7	8-Azabicyclo[3.2.1]octan-3-o
19	11.48	4,016,561	139,207	0.57	2.89	2(1H)-Naphthalenone
20	11.56	2,702,218	697,359	0.29	3.87	Megastigmatrienone
21	11.68	1,627,650	994,949	4.10	1.64	Cyclopentanone
22	12.02	3,482,475	672,212	0.28	5.18	Siglure
23	13.64	3,304,553	977,374	4.03	33.8	3-O-Methyl-d-glucose
24	13.98	1,609,296	657,505	2.71	2.45	Hexadecanoic acid
25	14.10	3,742,579	104,889	0.43	3.57	1,2-Benzenedicarboxylic acid
26	14.33	4,535,682	940,675	3.88	4.82	n-Hexadecoic acid
27	14.46	8,778,264	190,740	0.79	4.60	Uracil
28	15.14	2,926,962	166,331	6.85	1.76	9,12-Octadecadienoic acid (Z,Z)-
29	15.17	2,532,608	137,237	5.65	1.85	7,10,13-Hexadecatrienoic acid,
30	15.32	3,960,214	273,553	1.13	1.45	Phytol
31	15.36	2,107,588	142,080	0.59	1.48	Methyl stearate
32	15.60	2,477,771	257,140	10.5	9.64	9,12-Octadecadienoic acid (Z,Z)-
33	15.73	6,858,616	882,116	3.63	7.78	Octadecanoic acid
34	16.08	1,932,863	212,152	0.87	9.11	cis-5,8,11-Eicosatrienoic acid
35	16.20	1,514,668	170,056	0.70	8.91	Hexadecyl methanesulfonate
36	16.35	8,002,716	212,369	0.87	3.77	9,12,15-Octadecatrienoic acid
37	16.49	1,175,952	179,844	0.74	6.54	Fumaric acid
38	16.66	5,282,190	177,067	0.73	2.98	2-Dodecylcyclobutanone
39	16.71	2,591,615	108,790	0.45	2.38	Cyclohexanone
40	17.87	4,288,979	198,744	0.82	2.16	3-Cyclopentylpropionic acid
41	17.93	2,570,654	125,878	0.52	2.04	Carbamic acid
42	18.31	3,144,292	480,844	1.98	6.54	Hexadecanoic acid
43	18.66	3,653,137	153,763	0.63	2.38	Bis(2-ethylhexyl) phthalate
44	20.04	1,799,863	214,639	8.84	8.39	9,12,15-Octadecatrienoic acid,
45	20.21	8,529,886	196,384	0.81	4.34	Octadecanoic acid
46	20.96	1,308,486	461,131	1.90	2.84	Nonanedioic acid,
47	21.01	5,303,623	209,256	0.86	2.53	Nonanedioic acid
48	22.45	9,306,177	115,572	4.76	8.05	2,5,8-Metheno-2H-furo[4,3,2-ef][1]benzoxepine
49	22.64	4,387,856	150,951	0.62	2.91	Nonanedioic acid
50	22.71	2,697,445	801,538	0.33	3.37	4,5-Tetramethylenetetrahydro-1,3-oxazine
51	23.81	7,028,862	226,676	0.93	3.10	Vitamin E
52	24.37	1,735,591	500,335	0.21	3.47	26-Nor-5-cholesten-3.beta.-ol-25-one
53	24.51	3,149,951	911,905	0.38	3.45	9,19-Cyclocholestan-3-ol
54	24.99	5,065,730	141,690	0.58	3.58	Campesterol
55	25.29	1,830,334	681,892	0.28	2.68	Cholest-8(14)-en-3-ol

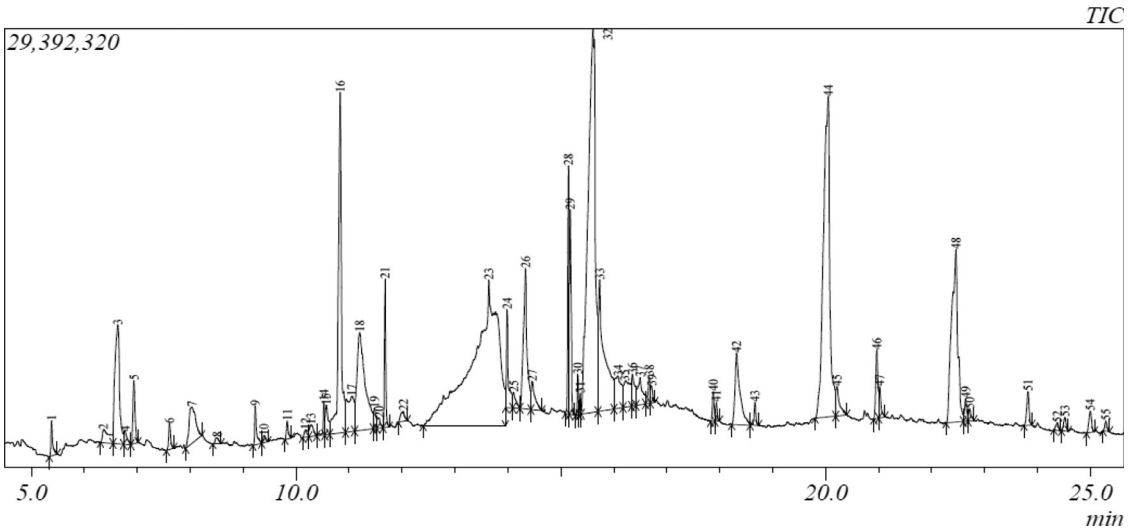


Fig. 1. Chromatogram of the bioactive compounds in methanol extract of *Trigonella foenum-greacum* seeds showing 54 peaks of the bioactive compounds.

58.31 mg TE/g (Table 3). The FRAP assay analyzes the reduction of Fe^{3+} to Fe^{2+} in a simpler, quicker, and more precise manner.

3.4. Cytotoxicity activity

MTT assay (Fig. 2) was used to measure the *in vitro* cytotoxic effect of *Trigonella foenum-greacum* seeds. Cell viability was calculated as a percentage for MCF-7 and fibroblast cells. The seed extract of *Trigonella foenum-greacum* in a concentration-dependent way shows a dramatically decreased in the viability of all malignant cells. While fibroblast cells showed a reduction in cell viability, there was a reduction in cell viability detected for the breast cancer cells. Cell viability was measured at 1000 g/ml as 67 and 86% for MCF-7 and fibroblast cells, respectively. However, the cell viability remains unchanged at lower concentrations, while a significant reduction was recorded only at 250 μg/ml and above.

3.5. Cell cycle and apoptosis

The cell cycle analysis result demonstrated a gradual proportional increase of cells arrested with increasing doses of the *Trigonella foenum-greacum* seed extract in both MCF-7 and fibroblast in the cell cycle. Specifically, exposure to SC_{10} and SC_{25} caused in the buildup of cells in the S-phase, with the proportion increasing from 31.2 ± 1.7 to 44.3 ± 2.19 and 54.6 ± 1.29%, respectively, in MCF-7 cells (Fig. 3). Furthermore, when treated with higher doses of the extract (SC_{35} and SC_{50}), cell cycle arrest was detected in a significant volume in the G_2/M checkpoint and $>G_2$ phases. The fraction of cells increased in the G2/M checkpoint (22.4 ± 1.4 to 77.23 ± 2.75%) and >G2 phase (0.0 ± 0.0 to 23.1 ± 2.89%) when treated with METFS at 100 μg/ml. Similarly, treatment with *Trigonella foenum-greacum* extract at 150 μg/ml resulted in 70.33 ± 3.59 and 30.83 ± 2.74% of cells being accumulated in G_2/M and $>G_2$ phase, respectively. Therefore, these results suggest that the *Trigonella foenum-greacum* seed extract suppresses cell proliferation by triggering dose-dependent cell at the S, G2/M checkpoint, and >G2 phases.

The necrosis and apoptosis data revealed that both the late and early apoptotic cells percentage changed in a dose dependent way. Fig. 4 illustrates that when cancer cells wete treated with 40 μg/ml of the extract, 13.3% of cells were necrotic but no apoptosis was observed. On the other hand, at the exposure of the cell to 80 μg/ml of the extract, 11.6% of the cell becomes necrotic, 8.81% were at early apoptotic, and 16.1% in delayed apoptotic. For cells exposed to the extract at 100 and 150 μg/ml, there was only about 5% necrosis. Apoptosis increased, with early apoptotic cells of 20.4 and 20.0% and 12.5 and 12.8% late apoptotic cells.

3.6. Mitochondrial membrane potential

Fig. 5 illustrates that TMRE fluorescence measured by flow cytometry displayed a considerable decrease in MMP in both cells at all designated dosages, in evaluation to the TMRE control, as well as between dosages. The MFI was calculated *via* flow cytometry for MCF-7 and fibroblast cells treated with METFS dosage of 0 (negative control), 40, 80, 100, and 150 μg/ml. The cells were grown to 2.5×10^5 cells in each well and treated with a given dosage in 6-well plates for 48 h.

3.7. Reactive oxygen species (ROS) generation

According to the results obtained**,** there was a significant increase in ROS at all dosages tested as compared to control, as indicated by the fluorescence of the DCFDA (Fig. 6). The MFI of DCFDA, as determined by flow cytometry, stayed calculated for MCF-7 and Fibroblast cells exposed to METFS at 0, 40, 80, 100, and 150 μg/ml.

4. Discussion

T. foenum-greacum, known as *Trigonella foenum-greacum*, is an herbal plant used as flavoring, supplements, and spices, either directly or after being dried. It has been reported to have various medicinal advantages in numerous studies. Breast cancer has been

Table 3
Extraction yield, DPPH, ABTS, TPC, TFC, and FRAPS summary.

Sample	Extraction yield (%)	SC_{50} of DPPH (ug/ml)	SC_{50} of ABTS (ug/ml)	FRAP	TPC (μg/g)	TFC (μg/g)
T. foenum-greacum seed	6.55 ± 0.05	19	11	58.31	128.3	14.93

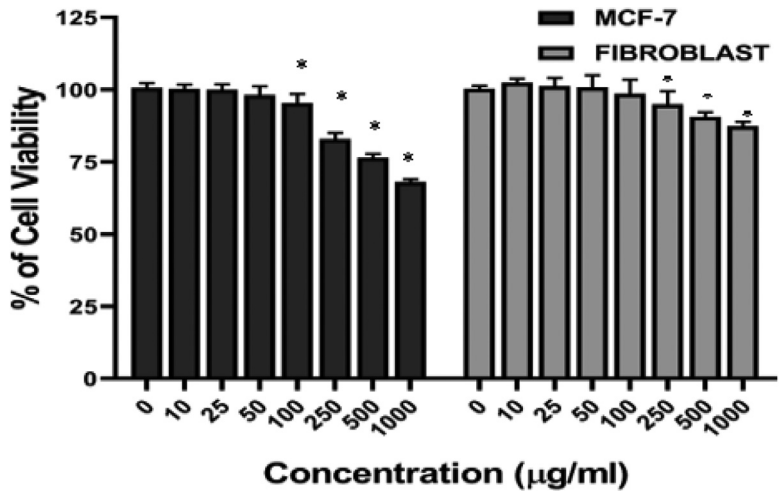


Fig. 2. Results of Cell Viability. MCF-7 showed a high decrease in cell viability, cell viability was measured at 1000 g/ml as 67% for MCF-7 and 86% for fibroblast. Cell viability was significantly reduced only at doses of 250 g/ml and above.

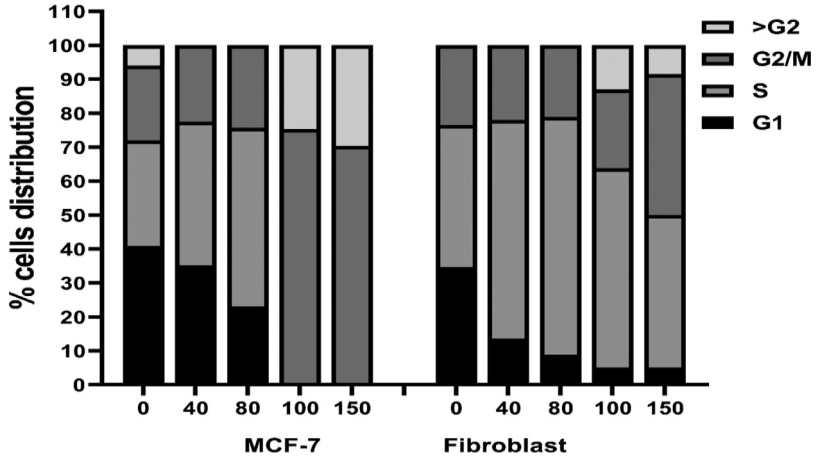


Fig. 3. Results of cell cycle distribution. The typical analysis of cell distribution in stages as ± SEM of triplicate different experimentations. The bar showed a reduction in G1 phase as the concentration increases indicating apoptosis in both MCF-7 and fibroblast. PI: Propidium Iodine.

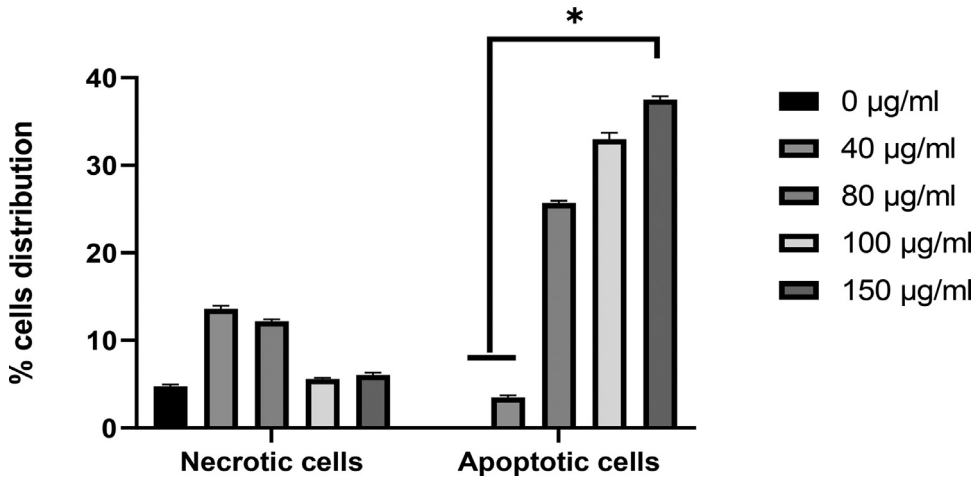


Fig. 4. Apoptosis and Necrotic cell results. In MCF-7 cell lines, the amount of necrosis was inversely proportional to the concentration of the extract, whereas apoptosis was directly related to the concentration of the extract.

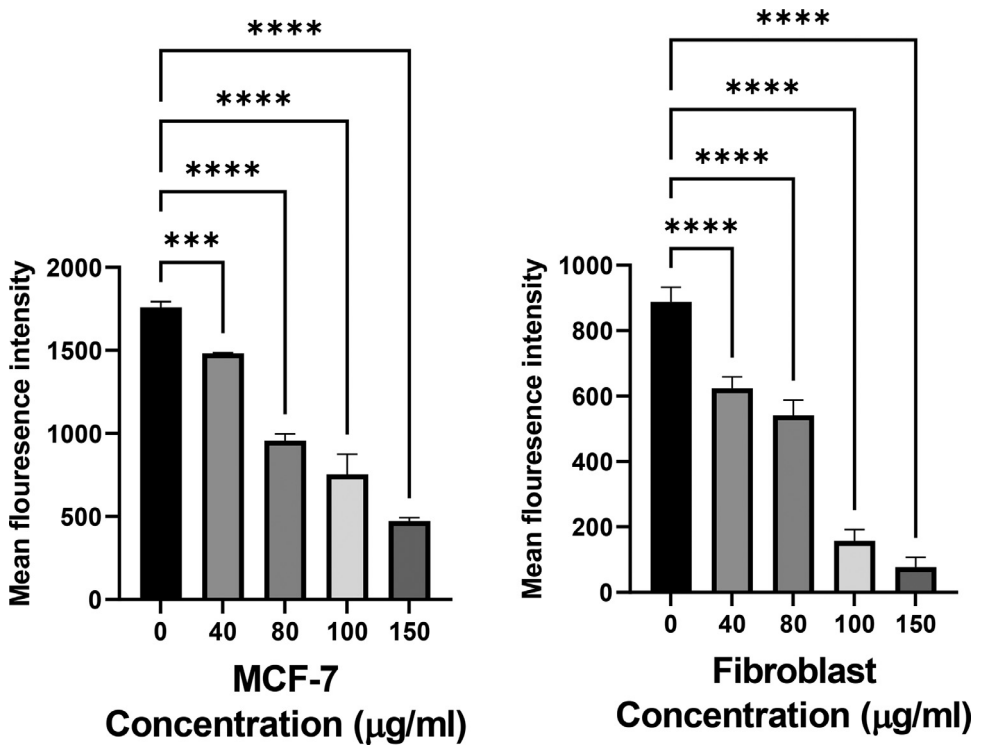


Fig. 5. Mitochondrial membrane potential results. Flow cytometry was used to quantify the m of the cells using TMRE. The mitochondrial membrane potential reduces as the concentration of the extract increases. TMRE, tetramethylrhodamine, ethyl ester.

reported as the most common cancer in women globally, accounting for 25% of all malignancies (Alwan, 2016; Azubuike et al., 2018). This study validates that the extract of *T. foenum-greacum* seed exhibits cytotoxic action that prevents MCF-7 cell development and agreed with the studies of Amin et al. (2005) and Kyung et al. (2006).

The study evaluated the antioxidant properties of *T. foenum-greacum* seed extract. The phytochemical screening results revealed that total flavonoid and phenolic compositions were higher than previously reported results (Aryal et al., 2019), confirming the plant's high antioxidant capacity. The ABTS assay showed stronger antioxidant activity than the DPPH assay. This agrees with earlier studies suggesting that the ABTS assay is more sensitive and leads to higher antioxidant activity results (Alsemari et al., 2014). The DPPH assay was employed to evaluate the ability of the extract to reduce stable DPPH radicals depending on the residual DPPH quantity (%) after 30 min (Bassermann et al., 2014). The study suggests that *T. foenum-greacum* seed extract has a significant antioxidant activity because of its high quantities of polyphenol chemicals.

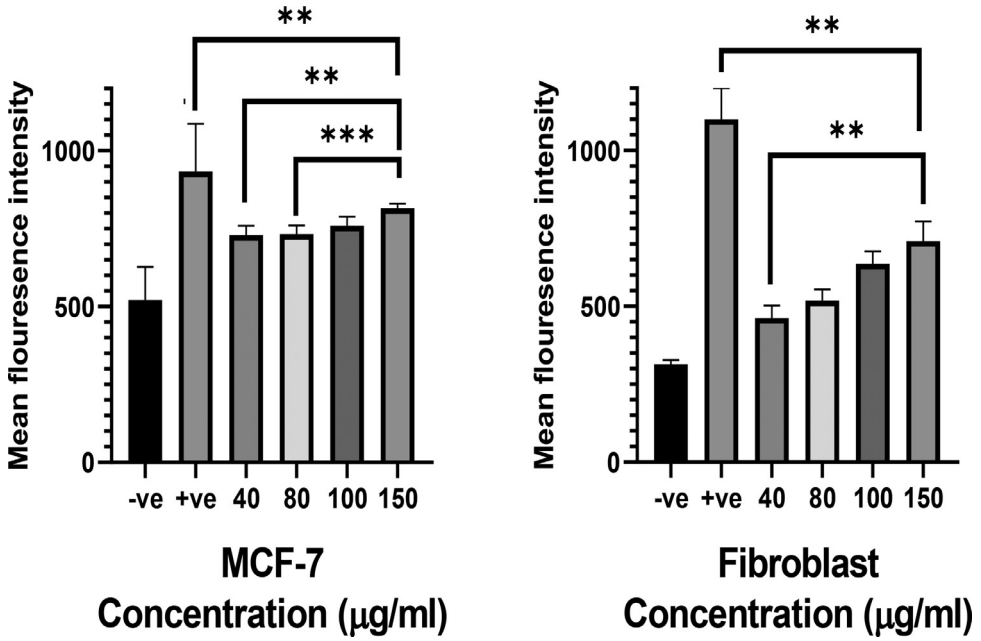


Fig. 6. Reactive oxygen species (ROS) generation as ± SEM of three separate studies. Data analysis were determined using GraphPad. *Significant difference versus METFS (+ve Control), **significant difference versus METFS 10 and 40 μg/ml, ***significant difference versus METFS 80 μg/ml. DCFDA, 2',7'-dichlorofluorescin diacetate.

The study found a variance in cell line sensitivity, as seen by the varied SC_{50} values of forty and one hundred and fifty gram per milliliters for Fibroblast and MCF-7 breast cancer cells, respectively, upon treatment with METFS. Earlier studies have suggested that METFS or diosgenin may cause apoptosis by activating Caspase-3, activating p53, and inhibiting Akts, but these results were not conclusive (Alshatwi et al., 2013; Kim et al., 2012). Using flow cytometry with FITC/PI, the necrosis percentage of late and early apoptosis was studied and shown to be dosage dependent. The late apoptosis proportion rose considerably in cells exposed to SC_{35} and SC_{50} dosages. The molecular mechanism of apoptosis was further elucidated by analyzing the cells distribution in the cell cycle. Interestingly, as seen in Fig. 3, METFS treatment resulted in G2/M, polyploidy and dose dependent S in cell cycle arrest. These results suggest that METFS induces cell cycle arrest in another way than earlier reported for different cancer cells.

Few reports shows the cell cycle arrest limit generated by plant extracts and isolated chemicals since cell life span is important in cancer development because it shows the spread of cell populations over time (Bassermann et al., 2014).

Our findings indicated a significant variation in the cell cycle. Specifically, treatment with SC_{10} and SC_{25} resulted in an accumulation of 55 and 56% in MCF-7 and Fibroblast cells respectively in the S-phase. During stress exposure, deoxy ribonucleic acid may be destroyed as cells struggle to reproduce it. The S-phase checkpoint is less sensitive than the G_1 or G_2/M phases and is thought not to arrest cell cycle progression, but rather to delay it (Barnum and O'Connell, 2014; Khoja et al., 2011). Our study results are interesting because they show that the behavior of cell cycle arrest altered dramatically with apoptosis-inducing dosages. Previous research has shown that blocking chromatin assembly and activating the p53-mediated mechanism can cause S-phase arrest (Fig. 4) (Chao et al., 2017; Estaquier et al., 2012).

As presented in Fig. 4, exposure to SC_{25} resulted in the detection of approximately 27 and 25% apoptotic cells in MCF-7 and Fibroblast, respectively. Intra- and extracellular stress can trigger the mitochondrial-associated apoptotic pathway, which relies on mitochondrial outer membrane permeabilization. Cytochrome C, released due to the loss of MMP, can activate the apoptotic machinery within cells (Badrinath and Yoo, 2018). Using flow cytometry and TMRE, we assessed the continuous decrease of MMP in both cells that had been treated to varied dosages of METFS (Fig. 5). Previous research has revealed that chemotherapeutic medicines can be selectively harmful *via* MMP pathways, as these pathways are important in oxidative signaling and ROS production (Nguyen and Pandey, 2019). In this study, it was discovered that a concentration dependent increases in ROS levels in both cells, which may be critical to METFS-induced apoptosis and cell cycle arrest (Fig. 6).

5. Conclusion

This study showed that methanolic extract of *T. foenum-greacum* seed displayed anticancer activity on MCF-7 cell lines by apoptosis or necrosis, reduction in membrane potentials and increasing ROS. This promising finding highlights the need to explore the use of *T. foenum-greacum* seed extract as a potential treatment for breast cancer.

Data availability

All data presented in this article are available with the authors.

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Nil.

Declaration of Competing Interest

Authors declare no conflicts of interest.

CRediT authorship contribution statement

Mutiu A. Alabi: Conceptualization, Methodology, Formal analysis, Resources, Investigation, Writing – original draft, Writing – review & editing, Supervision. **Marvis A. Arowolo:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing, Supervision. **Asiat Na'Allah:** Conceptualization, Formal analysis, Writing – original draft, Resources, Writing – review & editing, Supervision. **Pranav Kumar Prabhakar:** Conceptualization, Formal analysis. **Eberechukwuka G. Linus:** Conceptualization, Formal analysis. **Sesan A. Aransiola:** Conceptualization, Formal analysis. **Hassan T. Abdulameed:** Methodology, Investigation. **Beloved K. Ajani:** Methodology, Investigation. **Naga Raju Maddela:** Methodology, Investigation. **Ram Prasad:** Methodology, Investigation.

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