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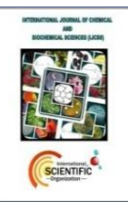

Mechanistic Insights: *Drynaria quercifolia* Rhizome Chloroform Fraction on S-Phase Cell Cycle Progression in Breast Cancer

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Abstract

Breast cancer is the most common gynaecological cancer in women, affecting more than 2.3 million women world-wide. Plant derived agents have been the most important source for identifying the leads in the development of anticancer drugs. The plant *Drynaria quercifolia* (DQ) is reported to be biologically active against various life affecting diseases including cancer. However, no extensive scientific investigations have been conducted to establish the anticancer activity of DQ. In this regard, the current study was carried out to investigate the anticancer properties of DQ Rhizome (DQR) fractions using bioassay guided fractionation. Successive soxhlet extraction of DQR was carried out with n-hexane, chloroform, ethylacetate and methanol, to get respective fractions. The *in vitro* anticancer activity of prepared fractions were assessed in Triple-negative breast cancer (TNBC) cells (MDA-MB-231). The cell viability, cell cycle and apoptosis were assessed using MTT assay, PI flowcytometry, and Annexin V-FITC/PI & AO/EtBr dual stain analysis respectively. Among the fractions, chloroform fraction showed a significant anticancer activity (CTC_{50} 31.70±0.84µg/ml) by interfering with the S-phase of the cell cycle. Further, chloroform fraction promotes early and late-stage apoptosis in TNBC MDA-MB-231 cells. The current investigation provides information on the anticancer activity of the DQR chloroform fraction.

Keywords: Apoptosis, Breast cancer, Cell cycle, *Drynaria quercifolia,* Rhizome

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1. Introduction

Triple-negative Breast cancer (TNBC) is one of the utmost aggressive and lethal cancer in women affecting more than 2.3 million people worldwide [1–3]. TNBC possess high rates of tumor relapse and metastatic features. Absence of molecular targets such as, HER2 (Human epidermal growth factor 2), ER (estrogen receptors) and PR (progesterone receptors) make the TNBC treatment difficult [4,5]. The primary risk factors includes age, genetic, hormonal changes, life style, reproductive and environmental factors [6,7]. Natural materials and their derivatives have a long history of usage in the treatment of numerous illnesses, including cancer [8,9]. Approximately 80% of global residents rely on plant-based medicines for their health care and currently more than 60% of natural derived agents are helpful in the treatment of cancer [10,11]. *Drynaria quercifolia* (DQ) is an epiphytic fern with a reduced root system attached to the host plant [12]. The plant profile is given in Table 1 [13–16] and there are different phytoconstituents with diverse pharmacological activity were reported for DQ Rhizome (DQR) [17–24], reported phytoconstituents were given in Table 2. However, no extensive scientific investigations have been conducted to establish the anticancer activity of DQ. In this regard, the current study was carried out to investigate the anticancer properties of DQR fractions using bioassay guided fractionation.

2. Materials and Methods

2.1. Cell line, culture condition and reagents

The National Centre for Cell Sciences (NCCS) in Pune, India, provided MDA-MB-231 triple-negative human breast cancer cells and HEK 293 human embryonic kidney cells. The cells were grown at 37 °C in a humidified atmosphere of 5% CO2 in F12 and DMEM (Gibco, USA) supplemented with 10% (v/v) FBS, 100 U/ml penicillin, and 100 g/mL streptomycin. Cell culture grade chemicals included MTT (Sigma Aldrich, USA), RNase (HiMedia,

India), PI (Sigma Aldrich, USA), Annexin V FITC (BD Biosciences, USA) and AO/EtBr (Sigma Aldrich, India).

2.2. Collection and authentication of plant

DQR was collected from Manipal University, Mangalore, Karnataka, India, in January 2021. The plant material was authenticated by T. Rasingam, Scientist In-charge, Botanical Survey of India (BSI), Hyderabad, Telangana, India. A voucher specimen reference Ref.No. BSI/DRC/2021-22/Tech./Identification/163.

2.3. Fractionation of DQR

A total of 200 g of DQR dry powder was extracted using n-hexane, chloroform, ethylacetate and methanol (500 ml) in a series of Soxhlet extractions. The fractions obtained were filtered and evaporated at 40 degrees Celsius using a rotary evaporator under reduced pressure.

2.4. HPTLC fingerprinting analysis of DQR fractions

CAMAG high performance thin layer chromatography (HPTLC) analyzer (CAMAG, Muttenz, Switzerland) was used to commence the HPTLC fingerprinting analysis of DQR fractions. Using a CAMAG Linomat 5 applicator, ten microlitre (50 mg/ml) of each methanol-prepared fraction was spotted on a precoated silica gel TLC plate (60 F254, Merck, Darmstadt, Germany). TLC was developed in a CAMAG twin trough development chamber with the appropriate mobile phases (Table 3). After development, the TLC plates were dried and scanned at 254 nm and 366 nm with the CAMAG HPTLC Scanner III and WIN CATS software (version 4.3). Following scanning, the plates were derivatized with anisaldehyde sulfuric acid reagent and imaged at 520 nm once again.

2.5. In vitro cytotoxicity and anticancer activity of DQR fractions

The cytotoxicity study and *in vitro* anticancer potential of DQR fractions were carried out using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium (MTT) assay. The cytotoxicity study of DQR fractions (0-1000 µg/ml) were carried out against HEK 293 cell line. All the fractions showed a CTC50> 500 µg/ml. Therefore, the DQR fractions with concentration range of 0-500 µg/ml were used for anticancer study against MDA-MB-231 cells. HEK 293 & MDA-MB-231 cells were seeded (1 x 10^4 cells/well) into 96-well culture plates and incubated at 37 °C for 24 hours with 5% CO2 and 95% air. After a 24-hour incubation period, the cells were exposed to various doses of DQR fractions. 20 µl of MTT reagent (5 mg/ml) was added after 48 hours of treatment and incubated for 3 hours. The resultant formazan was dissolved in 100 µl of DMSO. Finally, the absorbance at 570 nm was measured with a microplate reader (Tecan-Spark, Tecan Austria GMBH, Austria).

The percent cell viability was calculated by using the following formula:

$$\text{Cell viability} = (\text{OD treatment}/\text{OD control}) \times 100$$

Based on the significant activity, further the anticancer property of chloroform fraction was studied by using flowcytometry cell cycle analysis and cell apoptosis by using Propidium iodide (PI) staining, Annexin V-FITC (Flouorescein isothiocyanate)/PI & Acridine orange/ethidium bromide (AO/EtBr) dual stain on MDA-MB-231 cells.

2.6. Effect of chloroform fraction on cell cycle

PI stain and flow cytometry were used to examine the distribution of cell cycle phases. MDA- MB-231 cells were fixed at a density of 1×10^6 in a 6 cm culture plate and incubated for 24 hours. After 24 hours, cells were exposed to different concentrations (10, 20 & 50 µg/ml) of chloroform fraction for 48 hours. After incubation, cells were collected, washed with phosphate-buffered saline (PBS), and fixed for 30 minutes on ice in 1ml of 70% ice-cold ethanol. The cells were rinsed with PBS before being exposed for 1 hour at 37 °C with 15 µl of RNase A (0.1 mg/ml). Cellular DNA was stained with PI (10 µl, 40 µg/ml) and incubated at room temperature for 30 minutes in the dark. CyFlow Space (Partec, USA) flowcytometry was used to determine the DNA content and the BD FACSD software was used to calculate the percentage of DNA dispersion in the G0/G1, S & G2/M phases.

2.7. Effect of chloroform fraction on apoptosis

The proapoptotic activity of chloroform fraction was assessed in TNBC cells using Annexin V-FITC/ PI & AO/EtBr double staining assay.

2.7.1. Annexin V-FITC & PI double staining assay

The Annexin V-FITC/PI double satin technique is used to detect apoptotic cells (early apoptotic (EA) & late apoptotic (LA) cells). The assay was performed in accordance with the manufacturer's instructions (BD Biosciences, USA). MDA-MB-231 cells (1×10^6) were cultivated in a 6 cm culture plate for 24 hours before being treated with 10, 20 & 50 µg/ml of chloroform fraction for 48 hours. After the treatment period, the cells were collected and washed with cold PBS. The cell pellets were resuspended in Annexin V-binding buffer before being stained in the dark for 15 minutes at room temperature with 5 µl of annexin V-FITC and 5 µl of PI. Within 1 hour, flow cytometry was used to examine apoptotic cells.

2.7.2. AO/EtBr dual staining assay

In a 6-well culture plate, MDA-MB-231 (1×10^6) cells were seeded and incubated for 24 hours. Following incubation, the cells were treated with the appropriate doses of chloroform fraction (10, 20 & 50 µg/ml) and incubated for 48 hours. Following the incubation period, cells were washed twice with PBS, and 25 µl of AO (100 mg/ml) and EtBr (100 mg/ml) combination was added to cells and incubated at room temperature for 5 minutes. A fluorescent microscope was used to detect the stained cells.

3. Result

3.1. Fractionation of DQR

The percentage yield of DQR fractions were given in Table 4. Among the fractions, methanol fraction shows highest yield (1.52%).

3.2. HPTLC fingerprinting analysis of DQR fractions

The HPTCL densitogram spectrums of DQR fractions scanned at 254 nm, 366 nm and 520 nm (before and after derivatization with anisaldehyde sulphuric acid reagent) were given in Fig 3B & Table 5.

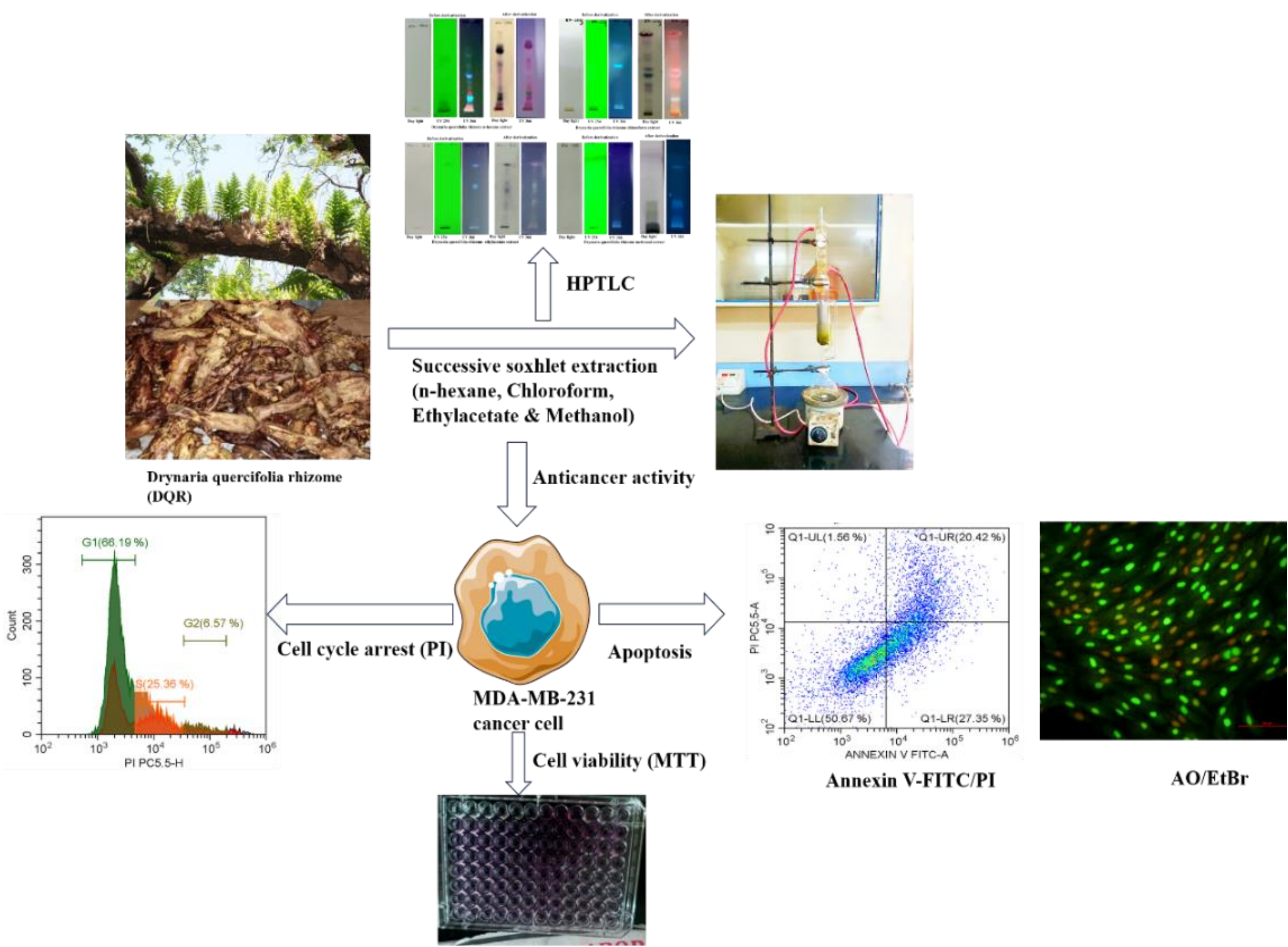

Figure 1.Graphical Abstract

Table 1. Plant profile – DQ

Kingdom	Plantae
Division	Pteridophyta
Class	Polyppodiopsida/pteridopsida
Order	Polypodiales
Family	Polypodiaceae
Subfamily	Polypodiaceae
Genus	Drynaria
Species	Quercifolia
Vernacular name	
English	Oakleaf fern
Tamil	Mudavattukal
Sanskrit	Aswakatri
Malayalam	Matipanna, pannakizhangu, pannikizhangu
Kannada	Hanuma hastha, hanuma paada
Hindi	Asvakatri, Katikapan, basingh.
Distribution	India, Bangladesh, Malysia, Southeast Asia, New Guinea, China, The Philippines, Pakistan, North America, Sri Lanka, Indonesia and Australia
Traditional uses	Typhoid, hectic fever, tuberculosis, asthma, jaundice, relieve head ache and throat infection, reduce cholera and urinary problems, reduce muscle pain, body pain, rheumatic pain and knee pain, and to restore hair growth

Table 2. List of Phytoconstituents isolated from DQ plant with their reported pharmacological activity

SI.NO	Name of the phytochemical	Structure	Pharmacological activity reported	Reference
1	Pentanoic Acid, Methyl Ester (PAME)		-	-
2	Undecane (UDC)		Anti-allergic, anti-inflammatory	[25]
3	Cyclohexasiloxane, Dodecamethyl- (CHDM)		Antifungal, antibacterial	[26,27]
4	1, 2-Benzenedicarboxylic Acid, Diethyl Ester (BDDE)		Plasticizer, antimicrobial	[28,29]
5	Cyclooctasiloxane, Hexadecamethyl- (COSHDM)		-	-
6	1, 3-Diphenyl-1, 3, 5, 5-Tetramethyl-cyclotrisiloxane (DPTMCT)		-	-
7	Benzenesulfonamide, 3-Amino-4-Hydroxy- (BSAH)		-	-
8	Octadecamethylcyclononasiloxane (ODMCS)		Antifungal, pesticide	[30,31]
9	Benzenepropanoic Acid, Alpha., 4-Bis (Acetyloxy)-, Methyl Ester (BPABME)		-	-
10	1, 2-Benzenedicarboxylic Acid, Bis (2-Methylpropyl) Ester (BDCBMPE)		Anticancer	[32]
11	2-Pyridinepropanamide, N-Phenyl- (PPNP)		-	-

12	Silane, [1, 3, 5-Benzenetriyltris (Oxy)] Tris [Trimethyl- (SBTTTM)		Antibacterial	[33]
13	Hexadecanoic Acid, Methyl Ester (HDME)		Antioxidant, anti-inflammatory, nematicide, insecticide, antiandrogenic, hemolytic, hypo – cholesterolemic, anticancer, antihistaminic, antieczemic, antiacne, antiarthritic, anticoronory	[34–36]
14	Palmitic Acid (PA)		Anti-inflammatory, antioxidant, hemolytic	[37]
15	1, 2-Benzenedicarboxylic Acid, Dibutyl Ester (BDCDE)		Antimicrobial, antifouling, antitumour agent	[38,39]
16	Nonamethyl, Phenyl-, Cyclopentasiloxane (NMPCPS)		-	-
17	1-Octadecanol (OD)		Antimicrobial	[28,38]
18	9, 12-Octadecadienoic Acid (Z, Z)-, Methyl Ester (ODDME)		Antibacterial, anticancer	[37,40]
19	9-Octadecenoic Acid (Z)-, Methyl Ester (ODZME)		Antioxidant, anticancer, anti-inflammatory, hypocholesterolemic, dermatigenic, anemiagenic	[34,36,38]
20	Tetracosamethylcyclododecasiloxane (TMCDS)		-	-
21	Octadecanoic Acid, Methyl Ester (ODME)		Antimicrobial	[34]
22	9-Octadecenoic Acid (OEOD)		Anticancer, anemiagenic, antiandrogenic, antitumour, dermatigenic	[41]
23	Pentamethyl Phenyl-Disilane (PMPDS)		-	-
24	(4-Chlorophenyl) Methanesulfonamide (CPMS)		-	-
25	1, 2-Benzenedicarboxylic Acid, Diisooctyl Ester (BDDIOE)		Antimicrobial	[42]

26	Phosphine Oxide, Triphenyl- (POTP)		-	-
27	3, 4-dihydroxy benzoic acid (DHBA)		Antibacterial, anti-viral, neuroprotective, anti-atherosclerotic, antifibrotic, anti-ageing, anti-ulcer, anticancer, anti-inflammatory, analgesic, antioxidant, cardioprotective, hepatoprotective, antihypertensive, bronchodilatory, antispasmodic	[43]
28	Naringin (NRGN)		Antidiabetic, cardioprotective, anti-inflammatory, antioxidant, neuroprotective, hepatoprotective, antitumour, antiviral, antibacterial, antiadipogenic, anticancer	[44–47]
29	2,4,6-cycloheptatrien-1-one,3,5-bistrimethylsilyl (CHBTMS)		Antioxidant	[48]
30	1,2-bis(trimethylsilyl) benzene (BTMB)		Antimicrobial, antioxidant	[49]
31	2-Propanaol, 1-chloro-3-isopropaxy- (PCIP)		-	-
32	Trimethyl-(4-trimethyl silylphenyl) silane (TTSPS)		-	-
33	3,5-Dichloro-4-(dodecylsulfanyl)-2,6-dimethylpyridine (DDSDP)		-	-
34	1-heptyn-4-ol (HPTN)		-	-
35	1-Chloro-3-propaxy-2-propanol (CPP)		-	-
36	2-myristynoyl-glycinamide (MGC)		Antimicrobial	[50]
37	tert-Butyl(2-isopropyl-5-methylphenoxy) dimethyl silane (TBDS)		-	-
38	3,4-dicarbomethoxybenzoic acid (DMBA)		-	-

39	Friedelin (FRDLN)		Antibacterial, antifungal, anti-inflammatory, analgesic, antipyretic, antihypertensive, antidiabetic, anticancer	[37,51–54]
40	Epifriedelinol (EFDRLN)		Anticancer	[55]
41	β-amyrin (BAMRN)		Gout, hyperpigmentation, analgesic, anti-inflammatory, anticonvulsant, antidepressive, hepatoprotective, antihyperglycemic, hypolipidemic	[56–58]
42	β-sitosterol (BSS)		Anticancer	[59,60]

Table 3. HPTLC mobile phase of DQR fractions

S. NO	Fractions	Mobile phase
1	n-hexane	Hexane: Ethylacetate (9:1)
2	Chloroform	Hexane: Ethylacetate (8:2)
3	Ethylacetate	Petroleum ether: Ethylacetate (8:2)
4	Methanol	Chloroform: Methanol (6:4)

Table 4. Percentage yield of fractions by soxhlation

S. No	Solvent	DQR (200g)	
		Yield (g)	Yield (%)
1	n-hexane	2.12	1.06
2	Chloroform	1.6	0.8
3	Ethylacetate	1.84	0.92
4	Methanol	3.04	1.52

Table 5. HPTLC fingerprint profile of DQR fractions

Track	Peak	Fractions											
		n-hexane			**Chloroform**			**Ethylacetate**			**Methanol**		
		Rf	**Area**	**Area%**	**Rf**	**Area**	**Area%**	**Rf**	**Area**	**Area%**	**Rf**	**Area**	**Area%**
254 nm	1	0.07	915	4.69	0.06	2685.6	8.29	0.06	563.8	1.70	0.05	3179.6	5.10
	2	0.12	406	2.08	0.24	915.9	2.83	0.08	179	0.54	0.22	13983.6	22.45
	3	0.15	676.1	3.46	0.30	796.6	2.46	0.13	209	0.63	0.36	646	1.04
	4	0.22	1589.3	8.14	0.40	7906	24.40	0.15	156.5	0.47	0.41	1383.3	2.22
	5	0.29	2856.6	14.63	0.49	446.8	1.38	0.18	460.6	1.39	0.57	6138.6	9.85
	6	0.52	13085.7	67.01	0.82	258.1	0.80	0.41	1456.7	4.40	0.69	15583.2	25.01
	7				0.88	626.8	1.93	0.49	1044.4	3.15	0.75	2277.1	3.66
	8				0.90	1624.7	5.01	0.59	3475.6	10.50	0.84	764.3	1.23
	9				0.94	17145.9	52.91	0.68	1584	4.78	0.92	18341.9	29.44
	10							0.80	326.5	0.99			
	11							0.93	15886	47.98			
	12							0.95	7769	23.46			
366 nm	1	0.06	279.8	0.45	0.1	2114.5	4.92	0.05	269.8	1.02	0.04	425.7	1.92
	2	0.08	470.9	0.75	0.19	11150.3	25.95	0.06	144.3	0.54	0.08	1288.3	5.82
	3	0.10	848	1.36	0.27	10215.8	23.78	0.09	839.6	3.16	0.23	2964.4	13.40
	4	0.17	39731	63.54	0.43	18468.3	42.99	0.19	1860.7	7.01	0.25	3435.3	15.52
	5	0.20	431	0.69	0.62	388.9	0.91	0.27	5198.2	19.59	0.37	1138.8	5.15
	6	0.25	14527.2	23.23	0.88	622.5	1.45	0.43	11800.5	44.48	0.45	1267.5	5.73
	7	0.34	1099.2	1.76				0.59	2124.6	8.01	0.50	443.3	2.00
	8	0.43	2165.5	3.46				0.75	1028.9	3.88	0.55	680.8	3.08
	9	0.47	1132.5	1.81				0.8	1523.4	5.74	0.62	5267.7	23.80
	10	0.64	232.2	0.37				0.85	1738.3	6.55	0.72	2404.4	10.87
	11	0.67	505	0.81							0.74	2813.4	12.71
	12	0.71	587.9	0.94									
	13	0.8	518	0.83									
520 nm	1	0.06	836.6	0.55	0.06	2441.4	1.77	0.09	613.3	0.94	0.07	4980	9.07
	2	0.1	6864.7	4.53	0.1	1178	0.85	0.18	6277	9.63	0.17	6333.9	11.54
	3	0.13	5044.2	3.33	0.17	6773.4	4.91	0.25	6463	9.91	0.25	6701.6	12.21
	4	0.18	306.7	0.20	0.2	2514.5	1.82	0.30	4962	7.61	0.41	390.7	0.71
	5	0.24	1225.3	0.81	0.25	14223.4	10.31	0.49	9860.1	15.12	0.49	985.1	1.79
	6	0.30	5013.2	3.30	0.30	6324.1	4.58	0.60	26110.8	40.05	0.57	1618.4	2.95
	7	0.36	25846.7	17.04	0.36	3175.4	2.30	0.93	8101.5	12.43	0.70	14980	27.29
	8	0.49	74380	49.03	0.38	3499	2.54	0.95	2810.5	4.31	0.76	9407.9	17.14
	9	0.72	32171.5	21.21	0.49	23017.4	16.68				0.87	207.5	0.38
	10				0.58	44998.6	32.62				0.91	666	1.21
	11				0.73	1057.1	0.77				0.94	8620.8	15.71
	12				0.78	3080	2.23						
	13				0.82	401	0.29						
	14				0.87	2027.1	1.47						
	15				0.89	2653.8	1.92						
	16				0.93	20596.8	14.93						

Table 6. CTC_{50} values of DQR fractions

Fractions	**CTC_{50} (µg/ml)**	
	Hek 293	**MDA MB 231**
n-hexane	523.05±3.46	54.77±0.99
Chloroform	518.85±4.87	31.70±0.84
Ethylacetate	552.00±4.52	69.55±1.30
Methanol	540.50±2.54	62.73±1.42

Fig2. Images of DQR and DQ leaf (DQL)

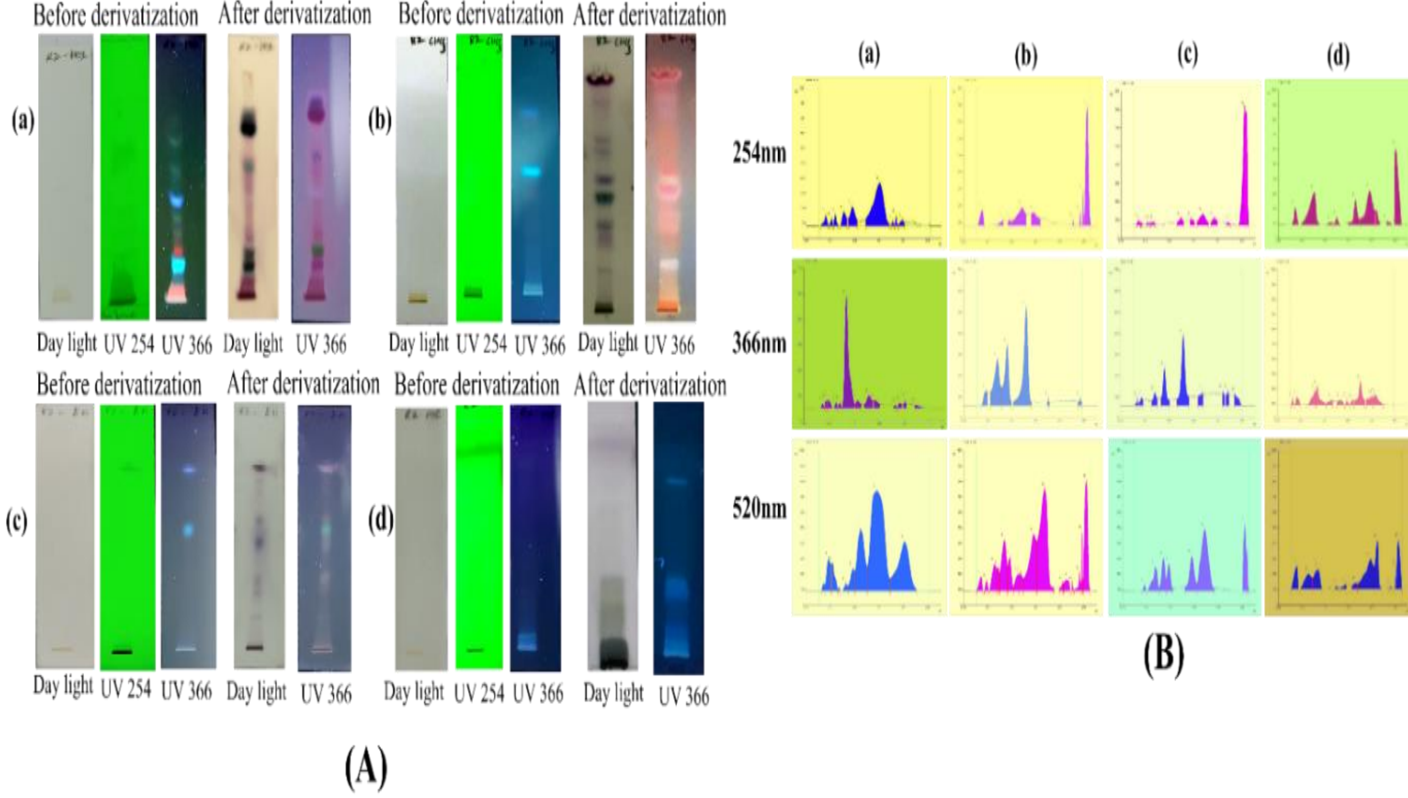

Fig 3. (A)

Chromatograms of DQR fractions, before derivatization: under day light, UV 254 nm and UV 366 nm; after derivatization: under day light and UV 366 nm (B) HPTLC Finger print of DQR fractions scanned at 254 nm, 366 nm and 520 nm (a) n-hexane fraction (b) Chloroform fraction (c) Ethylacetate fraction (d) Methanol fraction.

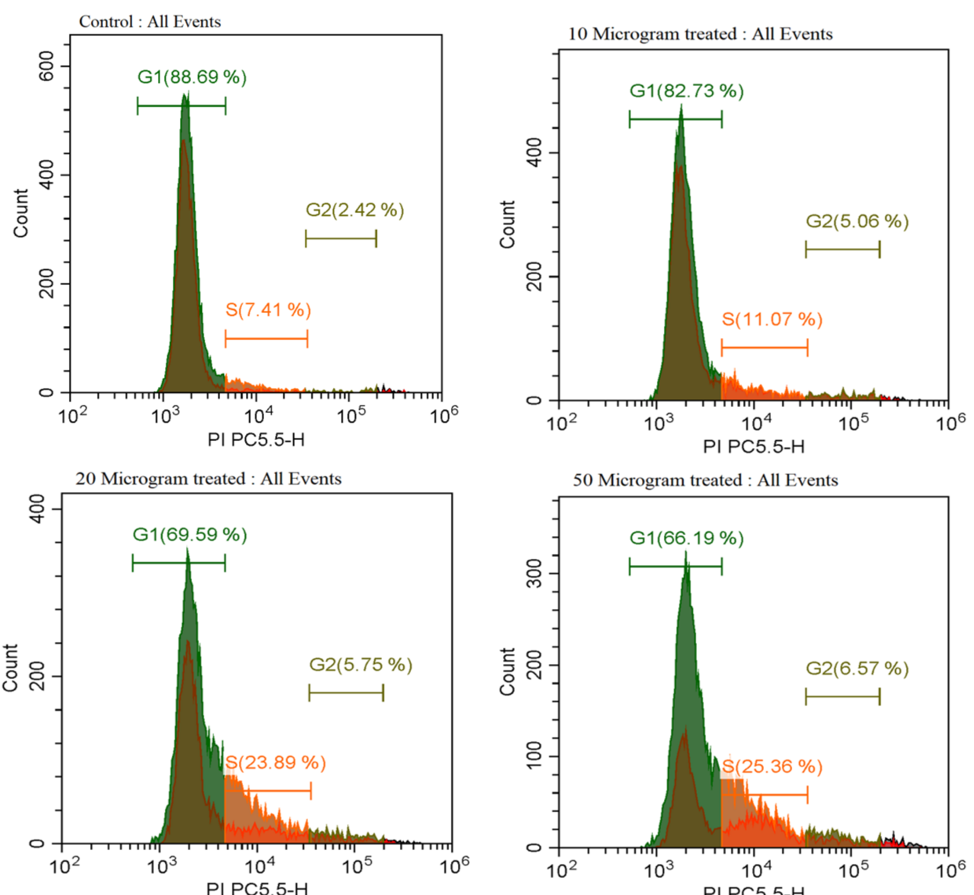

Fig 4. Effect of chloroform fraction on MDA-MB-231 cell cycle

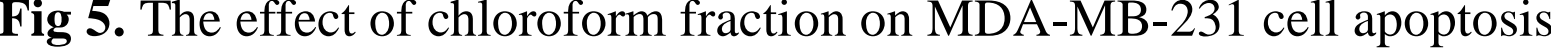

Fig 5. The effect of chloroform fraction on MDA-MB-231 cell apoptosis

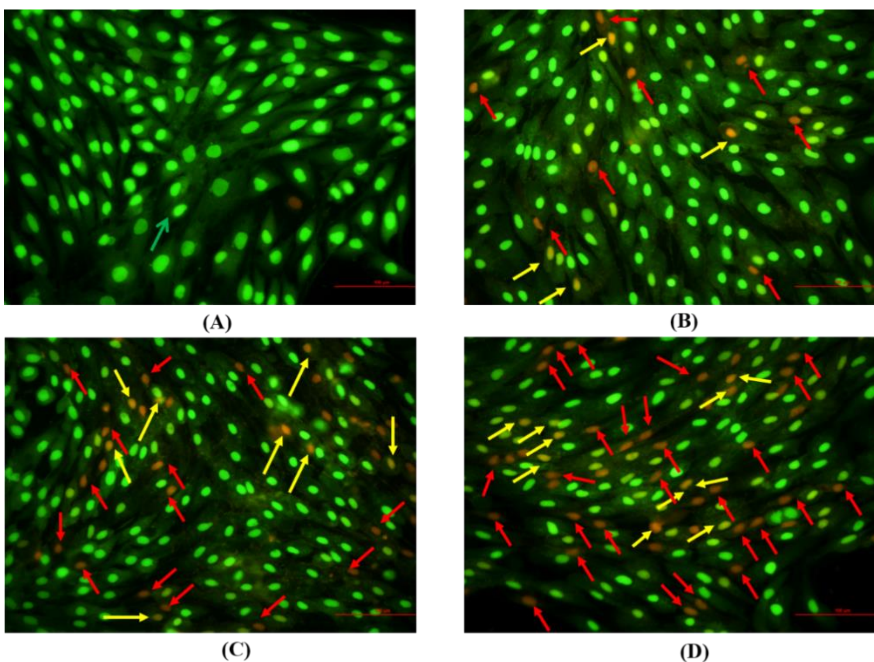

Fig 6. AO/EtBr double staining effect of chloroform fraction on MDA-MB-231 cells apoptosis. Green arrows represent live cells; yellow arrows represent EA cells and red arrows represent LA cells (A) Control (B) 10 µg/ml (C) 20 µg/ml (D) 50 µg/ml

Among the scanned fractions at 254 nm, the ethylacetate fraction shows highest peaks (12), followed by chloroform (9), methanol fractions (9) and n-hexane (6). Among the scanned fractions at 366 nm, the n-hexane fraction shows highest peaks (13), followed by methanol fraction (11), ethylacetate fraction (10) and chloroform fraction (6).

Among the scanned fractions at 520 nm, the chloroform fraction shows highest peaks (16), followed by methanol fraction (11), n-hexane fraction (9) and ethylacetate fraction (8) respectively.

3.4. In vitro cytotoxicity and anticancer activity of DQR fractions

Table 6 represents the *in vitro* cytotoxicity and anticancer activity of DQR fractions on Hek 293 and MDA-MB-231 cells. All the fractions showed a CTC_{50}> 500 µg/ml against Hek 293 cells, among the fractions, chloroform fraction exhibited significant anticancer activity with an CTC_{50} value of 31.70±0.84 µg/ml followed by n-hexane fraction (CTC_{50} 54.77±0.99), methanol fraction (CTC_{50} 62.73±1.42) and ethylacetate fraction (CTC_{50} 69.55±1.30).

3.6. Effect of chloroform fraction on apoptosis

3.6.1. Annexin V-FITC /PI double staining assay

Fig 4 illustrates the findings of the Annexin V-FITC/PI double staining test. In both apoptotic stages, the chloroform fraction at doses of 10, 20 & 50 µg/ml demonstrated considerable proapoptotic activity when compared to untreated control cells. The percentage increase in EA and LA cells was determined to be 6.06% & 8.79% at 10 µg/ml, 28.18% & 17.37% at 20 µg/ml, 27.35% & 20.42% at 50 µg/ml respectively.

3.6.2. AO/EtBr double staining assay

The results of AO/EtBr dual staining were given in Fig 5. The chloroform fraction has shown a dose dependent proapoptotic activity in MDA-MB-231 cells by decrease of viable cells (green fluorescence) and increasing the proportion of cells undergoing EA & LA when compared with the untreated control cells. The proapoptotic activity was confirmed by characteristic features such as chromatin condensation and membrane blebbing (Fig 5). The nuclear structure of normal cells resulted in green fluorescence, whereas EA cells resulted with bright green fluorescence, by the interposition of AO with the fragmented DNA. The cell's undergoing LA was identified by binding of EtBr to denatured DNA which results reddish-orange color.

4. Discussion

Plant-based anticancer medicines have been a major source in the development of anticancer agents [10]. The current study was focused the anticancer mechanism of DQR fractions (n-hexane, chloroform, Ethylacetate, and methanol) on MDA MB 231 cells. Among the fractions, the chloroform fraction exhibited significant anticancer activity with a CTC_{50} value of 31.70±0.84 µg/ml (Table 6). The chloroform fraction showed a significant arrest of MDA-MB-231 cells at S-phase in a dose dependent manner (Fig 3) and a significant increase of EA and LA cell population (Fig 4 & 5). Among the phytoconstituents of DQ, around 42 phytoconstituents (although not directly isolated and reported) found to show anticancer activity in various *in vitro* models (Table 2). These phytoconstituents includes BSS, FRDLN, BAMRN, NRGN, BDCBMPE, DHBA, EFDRLN, HDME, BDCDE, ODDME, ODZME and OEOD [54,61–69].

The BSS is reported to exhibit significant cytotoxic activity in liver cancer cells (HepG2 & Huh7) via inducing apoptosis and by activating caspase-3 and caspase-9 [70]. According to the Zhao et al., the phytochemical BSS prevents cell proliferation & enhances apoptosis in SGC-7901 human stomach cancer cells through increased expression of pro-caspase-3 and Bax and by decreased expression of Bcl-2 [71]. BSS significantly induces the cell apoptosis and cell cycle arrest of G0/G1 phase in breast cancer MDA- MB-231 cells by decreasing the level of CDK4 & cyclin D1 and by increasing the level of p21/Cip1 and p27/Kip1 [72]. Chang et al., reported that FRDLN triterpenoid shows significant anticancer activity against human AML-196 leukemia cells by inducing apoptosis via upregulating the level of caspase-3, 8, 9 and by blockage of MEK/ERK and PI3K/AKT signalling pathways [73]. Subash-Babu et al., reported that FRDLN as a potential anticancer agent against human breast cancer by inducing apoptosis by regulating the increased level of Cdkn1a, pRb2, p53, Nrf2, caspase-3 expression and by decreased level of Bcl-2, mdm2 & PCNA expression [74]. According to Wen et al., the anticancer impact of BAMRN in liver carcinoma cells (Hep-G2) is mediated by induction of apoptosis, interruption of the cell cycle, and activation of the JNK and P38 signalling pathways [75].

Banjerdpongchai et al., reported that, the flavonoid phytochemical NRGN induces apoptosis in HepG2 cells via mitochondria-mediated stimulation of caspase-9 and caspase-8-mediated proteolysis of Bid [76]. The phytochemical NRGN inhibits the bladder cancer cell proliferation and induces G1 phase arrest by upregulation of p21 protein [77]. Ajay kumar et al., reported that, BDCBMPE isolated from Onosma bracteata Wall shows significant anticancer activity by induction of early apoptosis by interfering G0/G1 phase of human osteosarcoma (MG-63) cells via Akt/NF-κB/p53 pathways [78]. Tseng et al., revealed that DHBA had potent anticancer effect by inducing cell cycle arrest and death in leukaemia cells (HL-60) via decreased RB and Bcl-2 expression and increased Bax expression [79]. Yang et al., found that the phytochemical EFDRLN shown substantial anticancer effect against cervical cancer cells (C33A and HeLa) in a dose-dependent manner via triggering apoptosis by modulating the levels of pro-apoptotic and anti-apoptotic proteins [80]. The obtained EFDRLN from Aster tataricus and Vitex penduncularis showed significant cervical cancer activity via increased level of caspase-3,-8 & -9 and also by reducing the level of Bcl-2, -xL and survivin respectively [81]. Based on the above reports the anticancer activity of chloroform fraction of DQR may be attributed to the presence of anticancer phytoconstituents and through above discussed anticancer mechanism.

5. Conclusion

The present study makes an effort to find the anticancer mechanism of DQR fractions. Among the fractions the chloroform shows promising anticancer activity by interfering with S-phase of cell cycle and by promoting apoptosis. Through literature we have identified 42 phytoconstituents of DQ which may be responsible for this anticancer activity. Further investigation may-shred more insights into the anticancer activity of this fraction.

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Authors' Contributions

All authors contributed to data analysis, drafting and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of interest

The authors state that there is no conflict of interest.

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