Mechanism of anti-cancer activity of edible tuber *Amorphophallus paeoniifolius* (APTE) against breast cancer cells

5.1. Introduction

Ethnobotanical approach to drug discovery is quite old and can be traced from the seventeen century [1]. Medicinal plants as anticancer agents are gaining more importance in the present scenario [2-4]. Several novel compounds from plants are in the market or in clinical trials from both edible as well as non-edible sources [5-7]. Studies are ongoing to find additional effective drugs which show low side effects and are inexpensive [8-11]. Current studies show that molecular alterations in the cancer cells are the best option for cancer therapy [12]. Undiscovered lead compounds from medicinal plants may play a critical role in cancer therapy by alteration at the molecular level to treat different cancers. Understanding the molecular mechanism of plant-extract based therapies against breast cancer thus will be important as alternative treatment options in breast cancer. *Amorphophallus paeoniifolius* (Elephant Yam) is a folk medicinal plant of India used in Ayurveda, Siddha, and Unani medicine. This tuber is reported to have antitumor, cytotoxic and anti-inflammatory properties along with various other traditional treatment uses [13-15]. Despite its wide traditional medicinal application, negligible clinical study had been done [15]. The tuber portion of the plant is popular as a vegetable in Indian society. In this chapter, we characterize the molecular mechanism of anticancer activities of *A. paeoniifolius* tuber extract.

5.2. Classification of the plant

Kingdom: Plantae
Subkingdom: Tracheobionta
Superdivision: Spermatophyta
Division: Magnoliophyta
Class: Liliopsida
Subclass: Arecidae
Order: Arales
Family: Araceae
Genus: *Amorphophallus* Blume ex Decne.
Species: *Amorphophallus paeoniifolius* (Dennst.) Nicolson

5.3. Material and methods

5.3.1. Cell lines and reagents

The cell lines MCF-7 and MDA-MB-231 were purchased from NCCS Pune, India. All reagents used were the same as **section 4.3.1. of chapter 4** except, Annexin V-Cy3 Apoptosis Detection Kit purchased from Sigma Aldrich, USA.

5.3.2. Preparation of extracts

The fresh tuber of the plant *Amorphophallus paeoniifolius* (Dennst.) Nicolson (Elephant foot yam), locally called "ul kochu" was collected from Golaghat district of Assam, India and morphological identification of the specimen was done by taxonomist Dr. Pankaj Chetia, Dibrugarh University, Assam, India. The fresh tuber was extracted with 50% denatured ethanol as described in **section 4.3.2. of chapter 4** and dried powdered extract (named APTE) was stored at 4°C.

5.3.3. Cell culture

MCF-7 and MDA-MB-231 cell lines were maintained as described in **section 4.3.4. of chapter 4.**

5.3.4. Migration assay

This experiment was performed as depicted in **section 4.3.4. of chapter 4.** The two non-toxic concentrations of APTE used here were 10 and 20 µg/ml.

5.3.5. Adhesion assay

Adhesion assay was carried out as described in **section 4.3.5. of chapter 4.** The two non-toxic concentrations of APTE used here were 10 and 20 µg/ml.

5.3.6. Invasion assay

This experiment was performed as described in **section 4.3.6. of chapter 4.** The two non-toxic concentrations of APTE used here were 10 and 20 µg/ml.

5.3.7. DNA fragmentation assay

This assay was done as in **section 4.3.7. of chapter 4.** MCF-7 and MDA-MB-231 cells were treated with a cytotoxic concentration of APTE (125 µg/ml) for 0 and 48 hr.

5.3.8. Annexin V-Cy3 assay

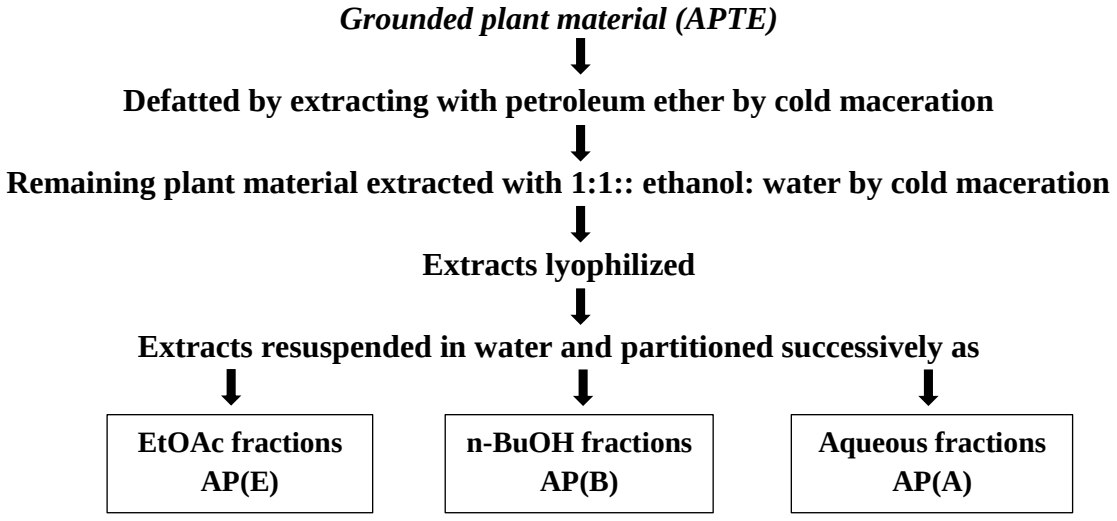
10,000 cells/well were plated in a 96 well plate and allowed to seed overnight. The cells were treated with APTE 125 µg/ml and 250 µg/ml followed by incubation for 24 hr. The media is removed carefully and Annexin V-Cy3 is added according to the manufacturer's protocol of Annexin V-Cy3 Apoptosis Detection Kit (Sigma Aldrich) and incubated for 10 min and fluorescent images were taken from five different fields of each well using fluorescence microscope (Model Olympus IX83, Japan).

5.3.9. Western blot analysis

Western blot was done as described in **section 4.3.10. of chapter 4.** In this case, we have used two non-toxic concentrations and two cytotoxic concentrations of APTE viz. 10, 20, 125 and 250 µg/ml to treat the cells. Doxorubicin 1 µg/ml was used as a positive control.

5.3.10. Fractionation of the extract

Successive fractionation of the extract was done similarly to **section 4.3.12. of chapter 4** to yield the EtOAc [AP(E)], n-BuOH [AP(B)] and aqueous [AP(A)] fractions. All the fractions were stored at 4^{o}C and checked for the biological activity.



5.3.11. Cytotoxicity assay using MTT

In this assay we have used MCF-7, MDA-MB-231, HT-29, and A549 cells for the analysis of either different fractions of APTE or crude RCFE as required and depicted

in **section 3.2.5. of chapter 3**.

5.3.12. Identification of bioactive compounds by LCMS

The crude extract of APTE was fractionated by the successive solvent fractionation method into ethyl acetate [AP(E)], n-Butanolic [AP(B)] and aqueous [AP(A)] fractions. Cytotoxicity of the dried fractions (125-1000 µg/ml) was assessed by MTT assay in both MCF-7 and MDA-MB-231 cells for 24 hr as described in **section 3.2.5. of chapter 3**. The active fractions were subjected to LCMS analysis in a HR-LCMS instrument (Agilent Technologies; Model 6550 Q-TOF LC/MS) for the identification of bioactive compounds at fragmentor voltage of 175 volts, for an acquired time of 30 min. The compounds were separated at 25° C on a Syncronis-C18 column (2.1 mm diameter, 1.7 µm particle size; Thermo Fisher Scientific). The injection volume was 5 µL. Elution was done in a gradient of water containing 0.1% HCOOH (A) and acetonitrile (B). The flow rate was 300 µL/min and the gradient was as follows: 0-5 min 5% B; 6–9 min 40% B; 10–15 min 50% B; 16–20 min 70% B; 21–25 min 90% B; 26–30 min 5% B. Contents of fractions were determined by the LC-ESI-MS/MS method.

5.3.13. Statistical analysis

Statistical analysis was performed using Graph Pad Prism and data were expressed as mean± standard deviation (mean± SD). Results were analyzed either by two-way analysis of variance (two-way ANOVA) or one-way analysis of variance (one way ANOVA) or Student's t-test as required by the experimental system and p-value >0.05 was considered non-significant (ns) and p-value <0.05 (***<0.0001; **<0.001and **<0.01) was considered significant.

5.4. Results

5.4.1. APTE inhibits migration of MCF-7 and MDA-MB-231 cells

Wound healing assay suggested that APTE treatment up to 48 hr inhibited the migration of both cells significantly in a dose-dependent way **(Fig 5.1.A & 5.1.B)**. While the wound widths were negligible in untreated cells, they were significantly high for APTE-treated cells suggesting the efficacy of the extract in migration inhibition.

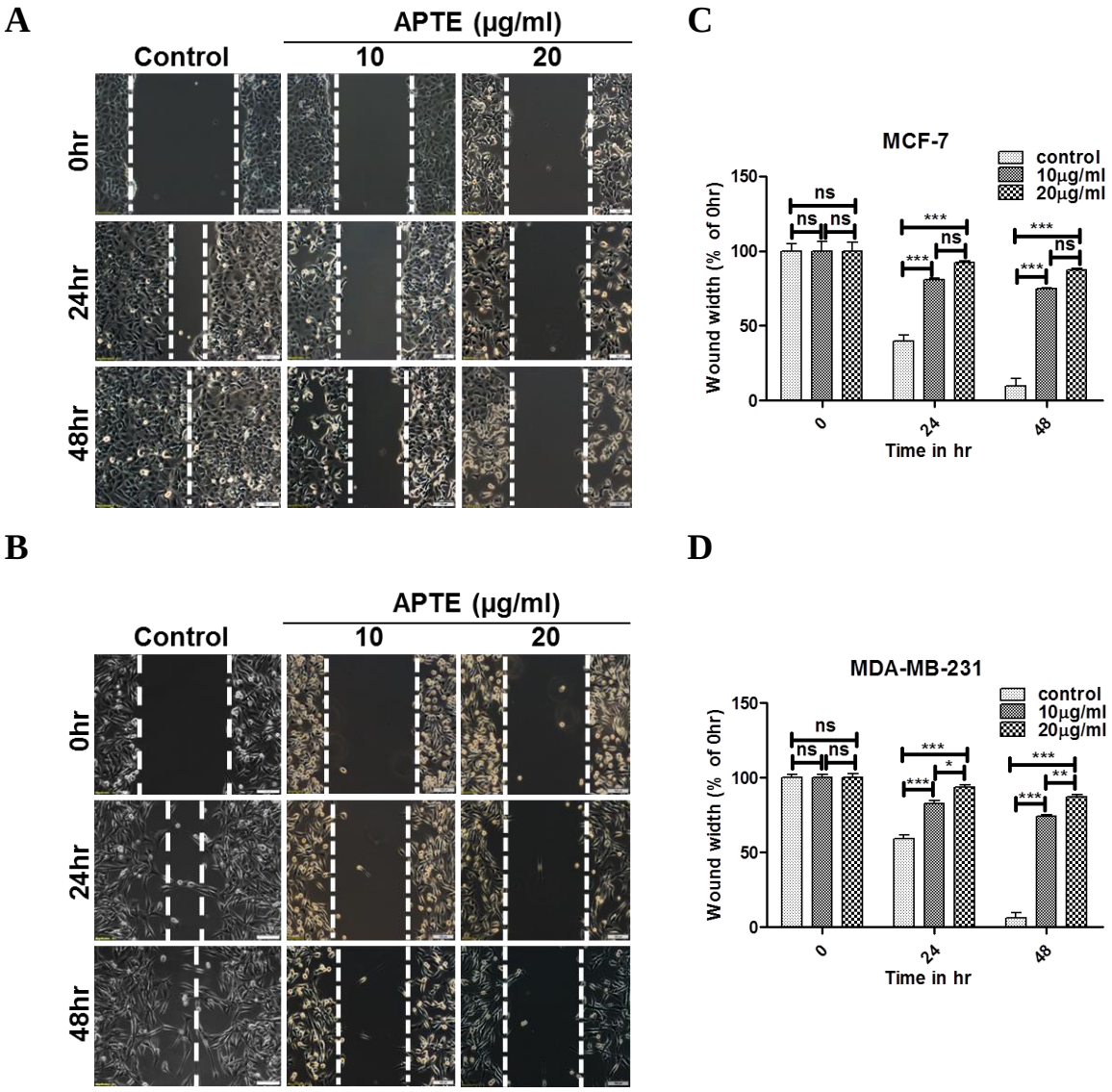


Figure 5.1.: APTE inhibited the migration of breast cancer cells. Inhibition of migration of MCF-7 **(A)** and MDA-MB-231 **(B)** cells treated with 10 and 20 µg/ml of APTE for 24 and 48 hr. The quantification of wound width as shown in the right panels **(C & D)**. Data represent the mean ± SEM of three independent experiments. Statistical differences were analyzed with two-way ANOVA test. p-value >0.05 was considered non-significant (ns) and p-value <0.05 (***<0.0001;**<0.001and **<0.01) was considered significant.

5.4.2. Adhesion of MDA-MB-231 to collagen IV is hindered by APTE

Pre-treatment of the cells with two concentrations of APTE suggested greater inhibition of adhesion in MDA-MB-231 cells compared to MCF-7 cells. APTE at 20 µg/ml concentration inhibited adhesion in MCF-7 cells by a moderate 19% **(Fig 5.2.A)**. In contrast, adhesion of the MDA-MB-231 cells was inhibited by 21% and 44% following treatment with 10 µg/ml and 20 µg/ml APTE, respectively **(Fig 5.2.B).**

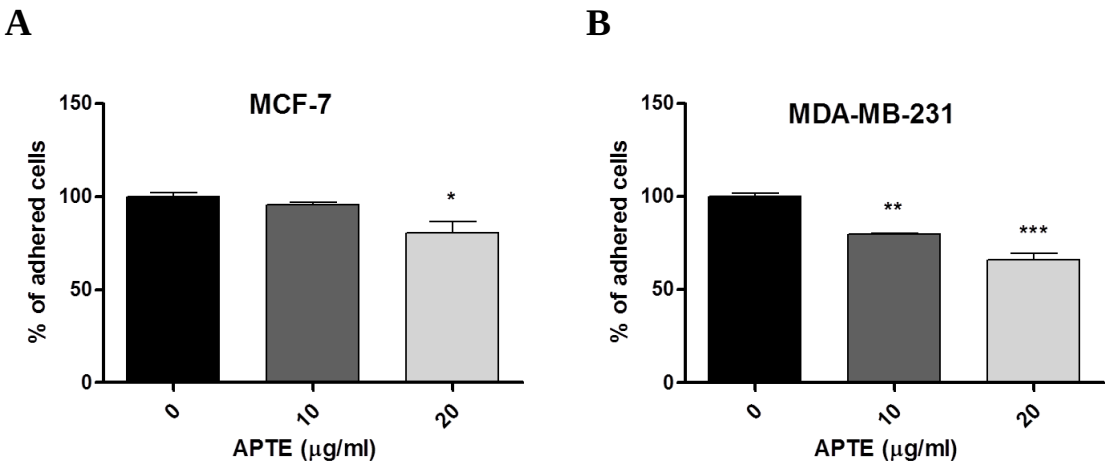
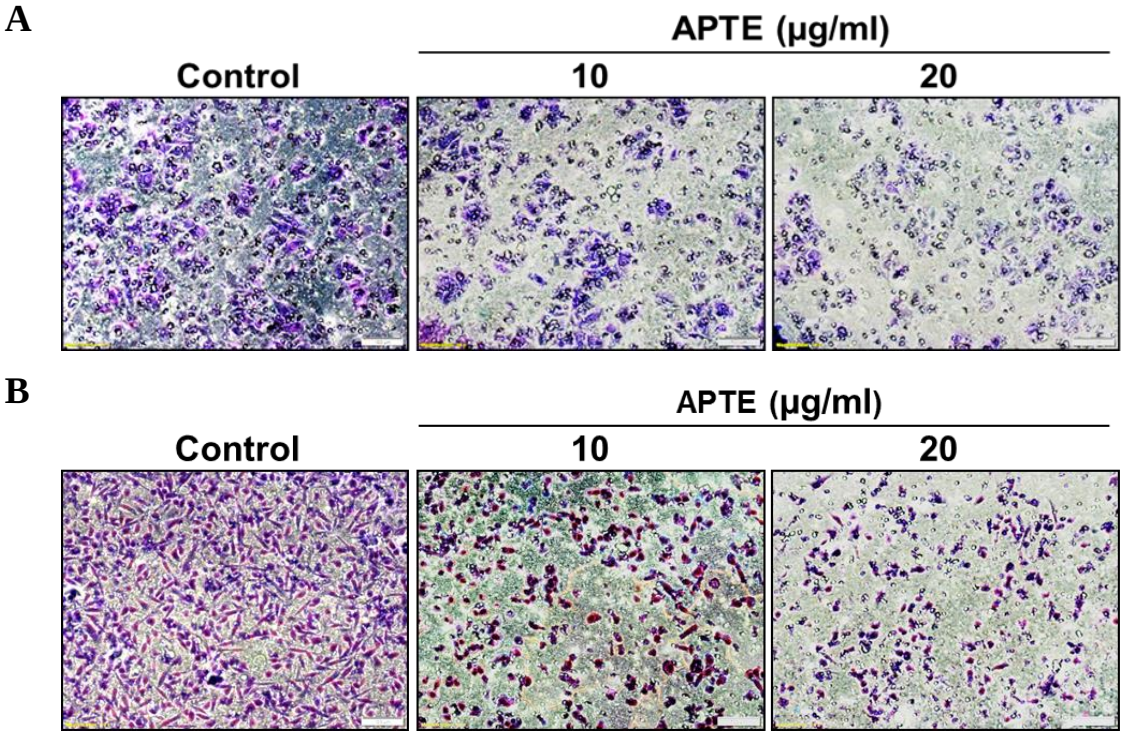


Figure 5.2.: Adhesion of MDA-MB-231 to collagen IV is hindered by APTE. Effect of APTE on adhesion of MCF-7 **(A)** and MDA-MB-231 **(B)**. Data represented as mean ± SEM of three independent experiments of cells adhered to collagen-coated wells and quantified by MTT. Statistical differences were analyzed with one-way ANOVA test. p-value >0.05 was considered non-significant (ns) and p-value <0.05 (***<0.0001;**<0.001and **<0.01) was considered significant.

5.4.3. Invasion of MDA-MB-231 cells is reduced by APTE

The inhibition in invasion in response to APTE treatment was not significant in MCF-7 cells **(Fig 5.3.A)**. Interestingly, the effect was highly significant in MDA-MB-231 cells, as treatment with 10 µg/ml and 20 µg/ml of APTE led to 65.5% and 73% inhibition in the invasion, respectively **(Fig 5.3.B)**.



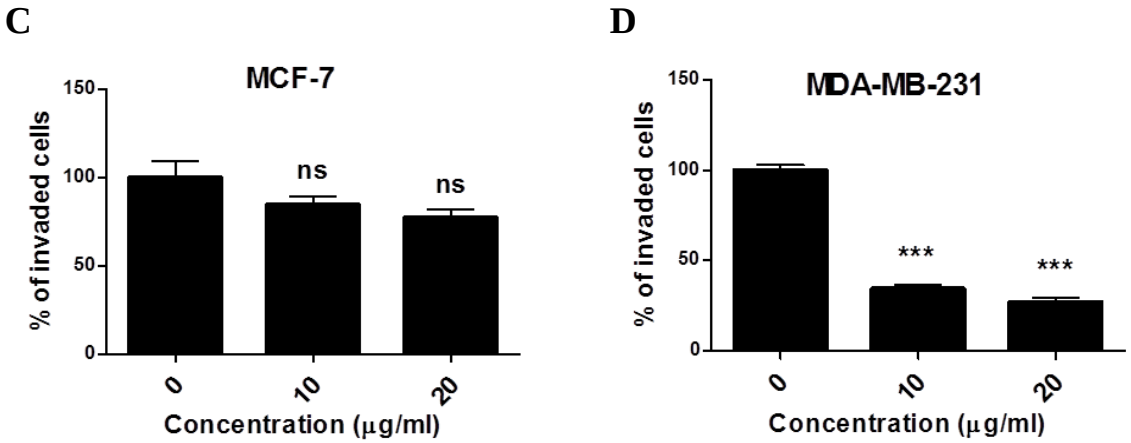


Figure 5.3.: Invasion of MDA-MB-231 cells is reduced by APTE. Invasion of MCF-7 **(A)** and MDA-MB-231 **(B)** cells through ECM gel-coated transwell inserts in response to APTE. Images of invaded cells after staining were shown (A & B). Data represent the mean ± SEM of five different images of individual set of three independent experiments **(C & D)**. Statistical differences were analyzed with student t-test. p-value >0.05 was considered non-significant (ns) and p-value <0.05 (***<0.0001;**<0.001and **<0.01) was considered significant.

5.4.4. APTE induced DNA degradation in MCF-7 and MDA-MB-231 cells

As apoptosis is a possible mode of cell death we studied DNA fragmentation in cells treated with APTE **(Fig 5.4.)**. Genomic DNA isolated from the cells following treatment of APTE (125 µg/ml) for 48 hr showed DNA degradation in both the cells and indicated the efficacy of APTE to induce apoptosis in both the cells.

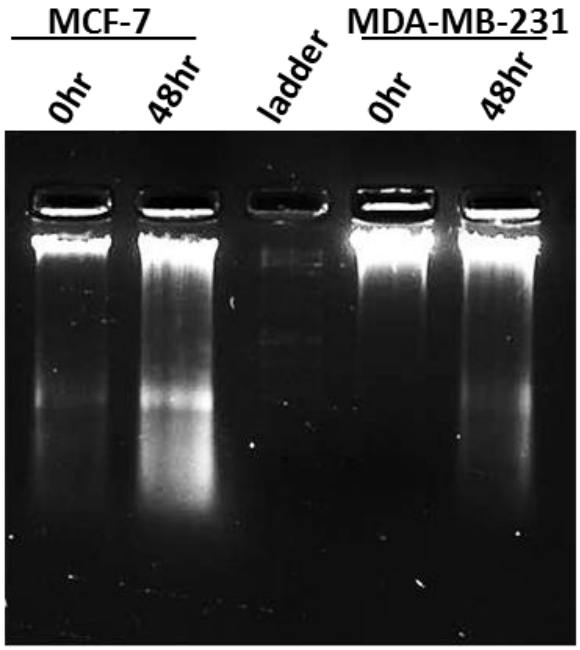
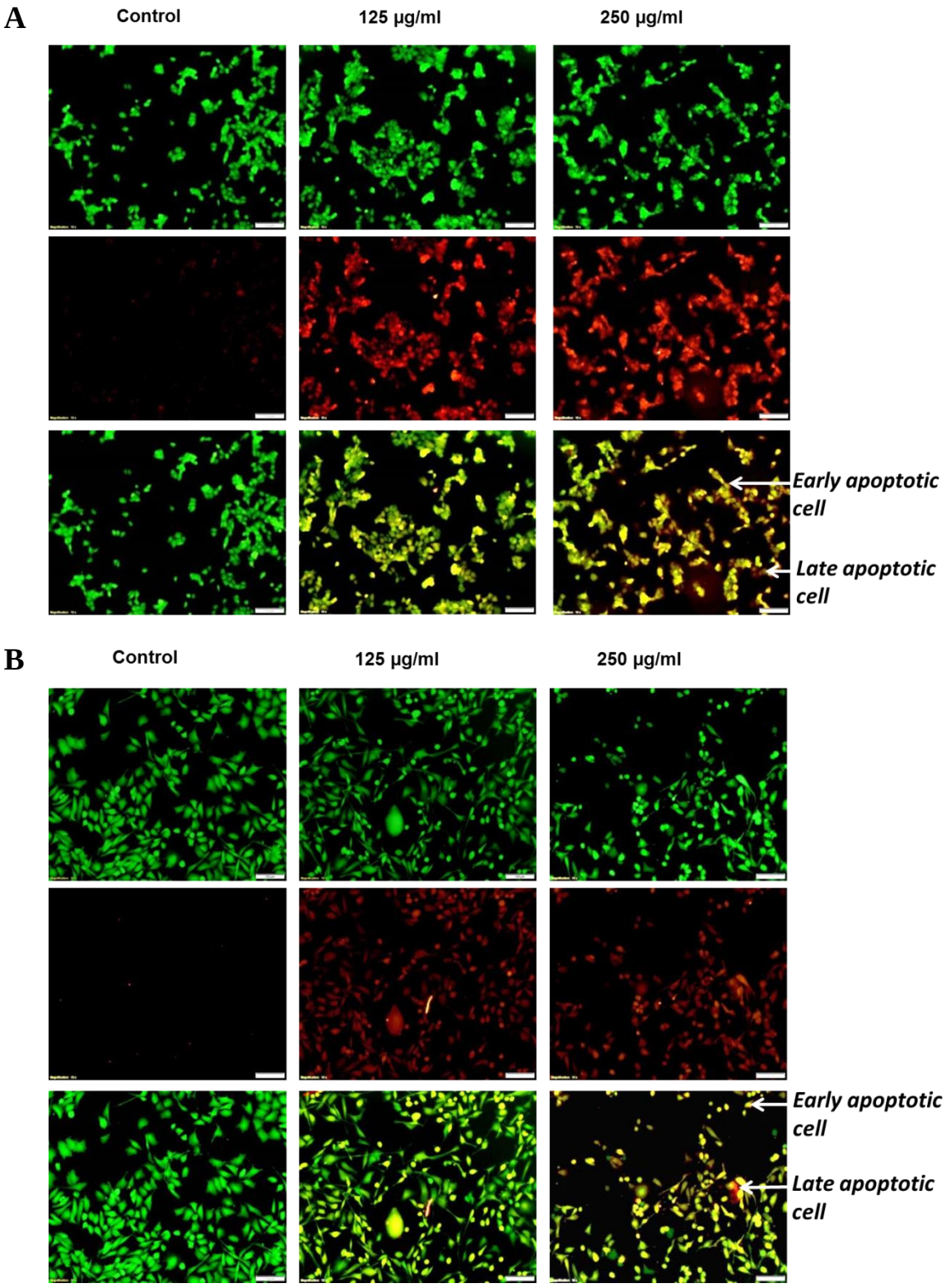


Figure 5.4: APTE induced apoptosis in MCF-7 and MDA-MB-231 cells. The DNA fragmentation was observed in APTE (125 µg/ml) treated MCF-7 and MDA-MB-231 cells in 24 and 48 hr on 2% Agarose gel electrophoresis.

5.4.5. Induction of apoptosis by APTE

To confirm apoptosis induction in both MCF-7 and MDA-MB-231 cells following APTE treatment, fluorescence-based Annexin V-Cy3 Apoptosis Detection Kit (Sigma



C **D**

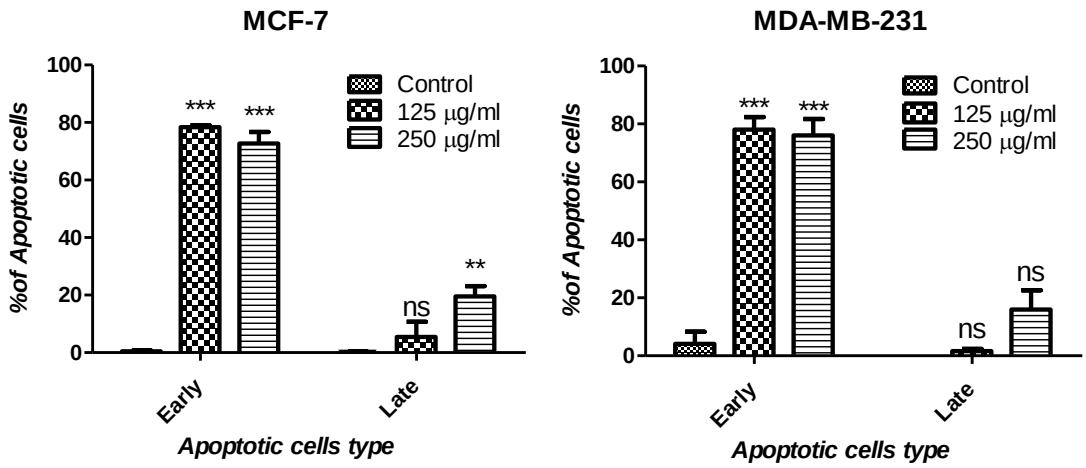


Figure 5.5.: Quantification of apoptotic cells by Annexin V-Cy3. Manual quantification of images of stained MCF-7 **(A)** and MDA-MB-231 **(B)** using Annexin V-Cy3. The quantitation of three independent analysis was presented in the right panels **(C & D).**

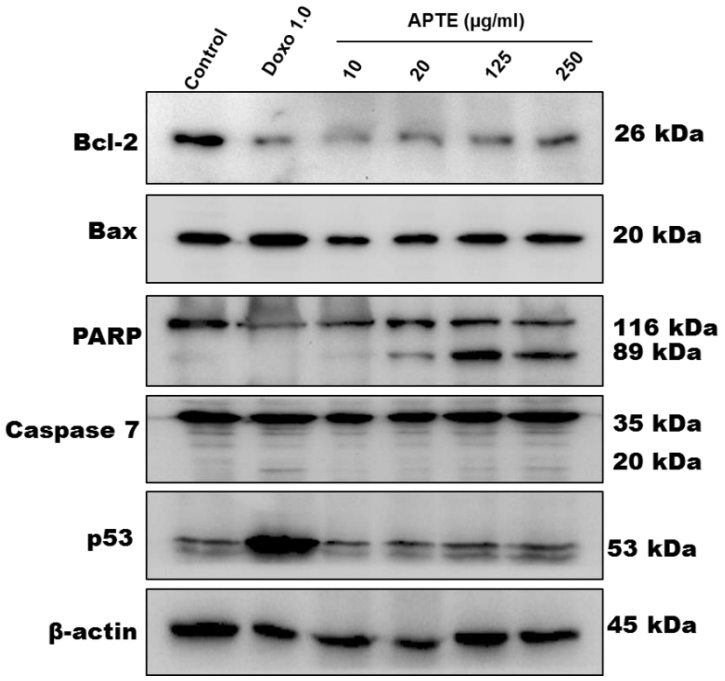
Aldrich) was used. After 24 hr of APTE treatment, fluorescent cells were counted manually using images from various fields of individual treatment. Cells in green denoted live cells while those in red and green (yellow when merged) indicated cells undergoing apoptosis. The red cells represent late apoptotic or necrotic cells **(Fig 5.5.A-B)** [16]. Quantification of the images showed that 0, 125 and 250 µg/ml of APTE treatment for 24 hr induced apoptosis in 0.7%, 84% and 92% in MCF-7 cells and 4%, 80% and 92% in MDA-MB-231 cells respectively **(Fig 5.5.C** & **D)**.

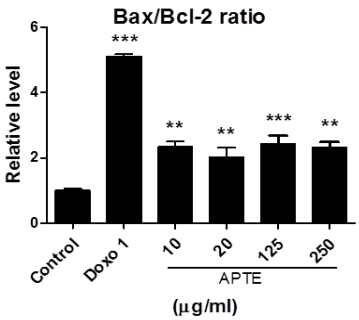
5.4.6. Western blot analysis of apoptosis regulating proteins

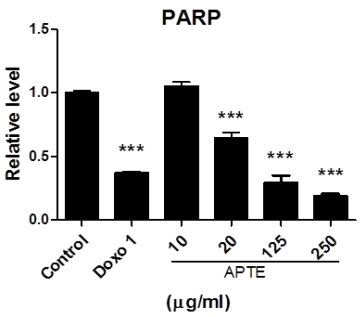
The apoptosis-inducing activity of APTE was confirmed by immunoblot studies of selected apoptosis-associated proteins **(Fig 5.6.A & B)**. Two low (10 µg/ml and 20 µg/ml) and two significantly high (125 µg/ml and 250 µg/ml) concentrations of the extract were selected for treatment. APTE inhibited expression of anti-apoptotic Bcl-2 and induced expression of pro-apoptotic Bax leading to augmentation of Bax/Bcl-2 ratio in both the cell lines **(Fig 5.6.A & B)**. Bax/Bcl-2 ratio was induced more than 5 folds compared to control in MCF-7 cells treated with doxorubicin (1 µg/ml) and ~2 folds with all experimental concentrations of APTE treatment. In contrast, the ratio did not change significantly in MDA-MB-231 cells treated with doxorubicin. However, a dose-dependent increase in the Bax/Bcl-2 ratio was found in the case of APTE treatment. The

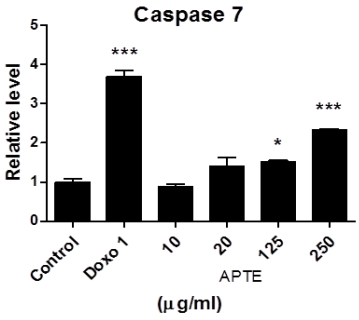
treatment with the extract induced PARP cleavage, a hallmark of apoptosis, in both cell lines. PARP cleavage was evident in MCF-7 cells in response to low concentration (10 µg/ml) of APTE, while higher concentration of APTE (125 µg/ml and above) was required for PARP cleavage in MDA-MB-231 cells. Further, it successfully induced Caspase-7 activation and cleavage in a concentration-dependent manner. However, expression of tumor suppressor protein p53 seems to be unaltered with increasing concentration of APTE in both the cell lines. Treatment with doxorubicin (1 µg/ml) upregulated expression of p53 in MCF-7 but not in MDA-MB-231 cells as it contains a mutant p53.

A









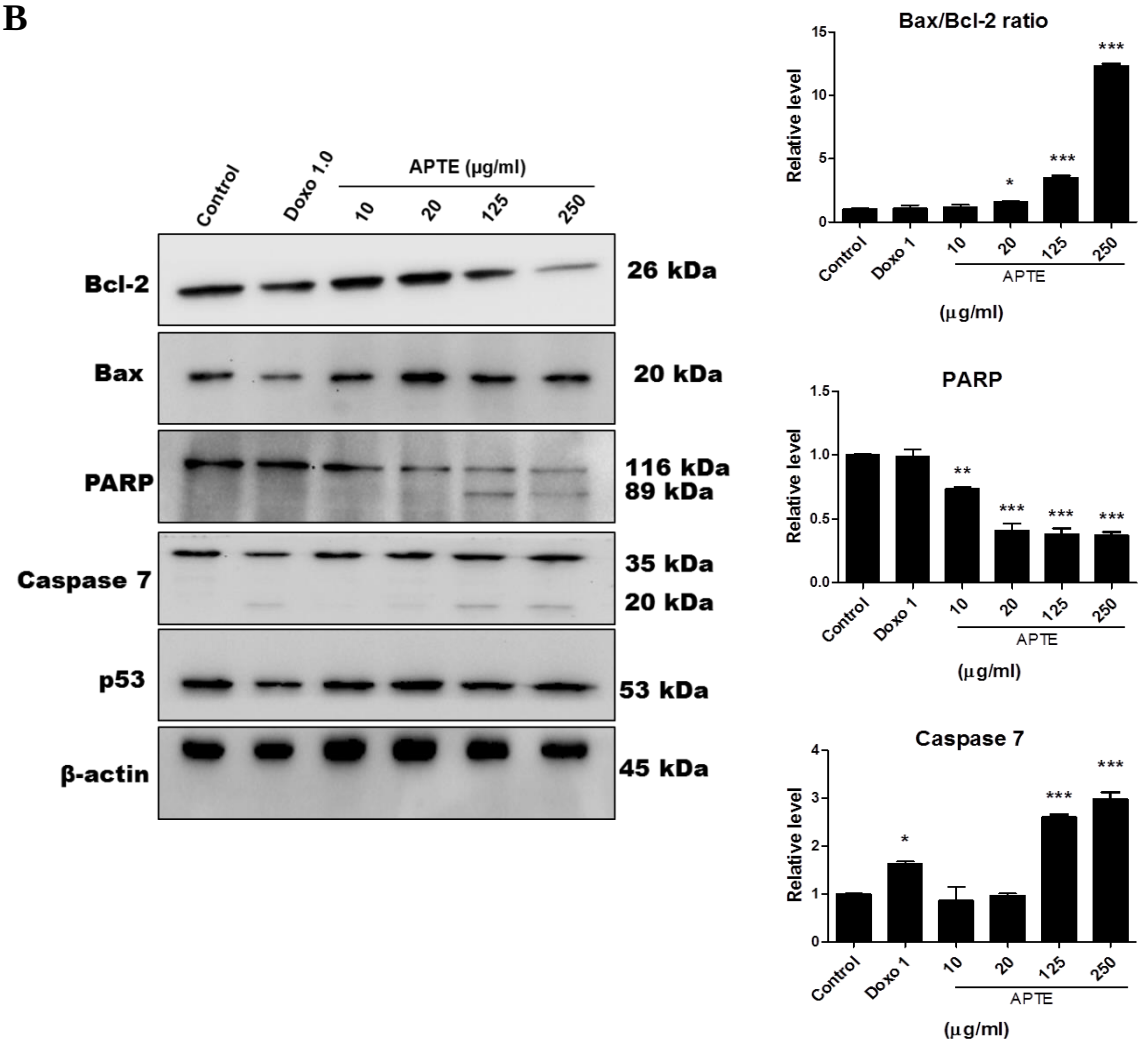


Figure 5.6.: Western blot analysis of induction of apoptosis in lysates from MCF-7 **(A)** and MDA-MB-231 **(B)** with antibodies against Bcl-2, Bax, PARP, Caspase-7, and p53. Doxorubicin (1µg/ml) was used as positive control. β-actin is used as loading controls. The ratio of Bax/Bcl-2, caspase-7, and p53 expressions was normalized to β-actin presented in the right panels. Statistical differences were analyzed with one-way ANOVA test. p-value <0.05 was considered significant.

5.4.7. Bioactive compounds in APTE

To identify the tentative compounds with a probable contribution to anticancer activity, APTE was fractionated successively into three solvents: ethyl acetate [AP(E)], n-butanolic [AP(B)] and aqueous [AP(A)] fractions. Amongst these three fractions, AP(E) and AP(B) showed significant cytotoxicity in both MCF-7 and MDA-MB-231 **(Fig 5.7.A & B)**. Treatment with 1000 µg/ml AP(E) and AP(B) for 24 hr induced 45% and 58% cytotoxicity in MCF-7 cells compared to control. Similar treatment induced 55% and 49% cytotoxicity in MDA-MB-231 cells. AP(A) fraction was not efficient as AP(E) and AP(B) fractions and therefore, latter two active fractions were subjected to HR-

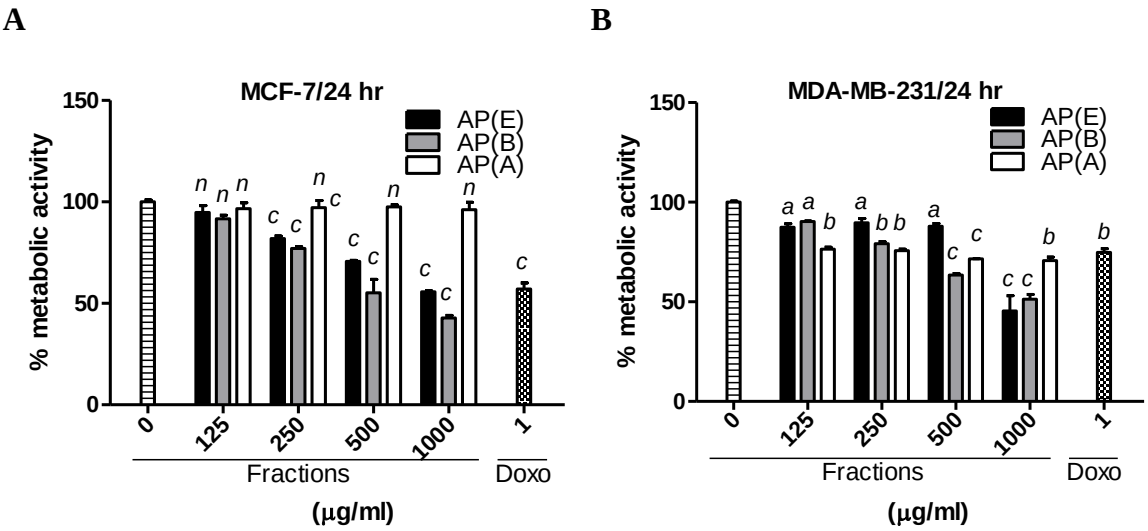


Figure 5.7.: Cytotoxic effect of various fractions of APTE on human breast cancer cells. Cytotoxic activity of ethy-lacetate [AP(E)], butanolic [AP(B)] and aqueous [AP(A)] fractions APTE on (A) MCF-7 and (B) MDA-MB-231 cells. Doxorubicin (1µg/ml) was used as a positive control. Data represent the mean ± SD of three independent experiments. Statistical differences were analyzed with two-way ANOVA test. (p values for a < 0.05, b <0.01, c <0.001 and n > 0.05).

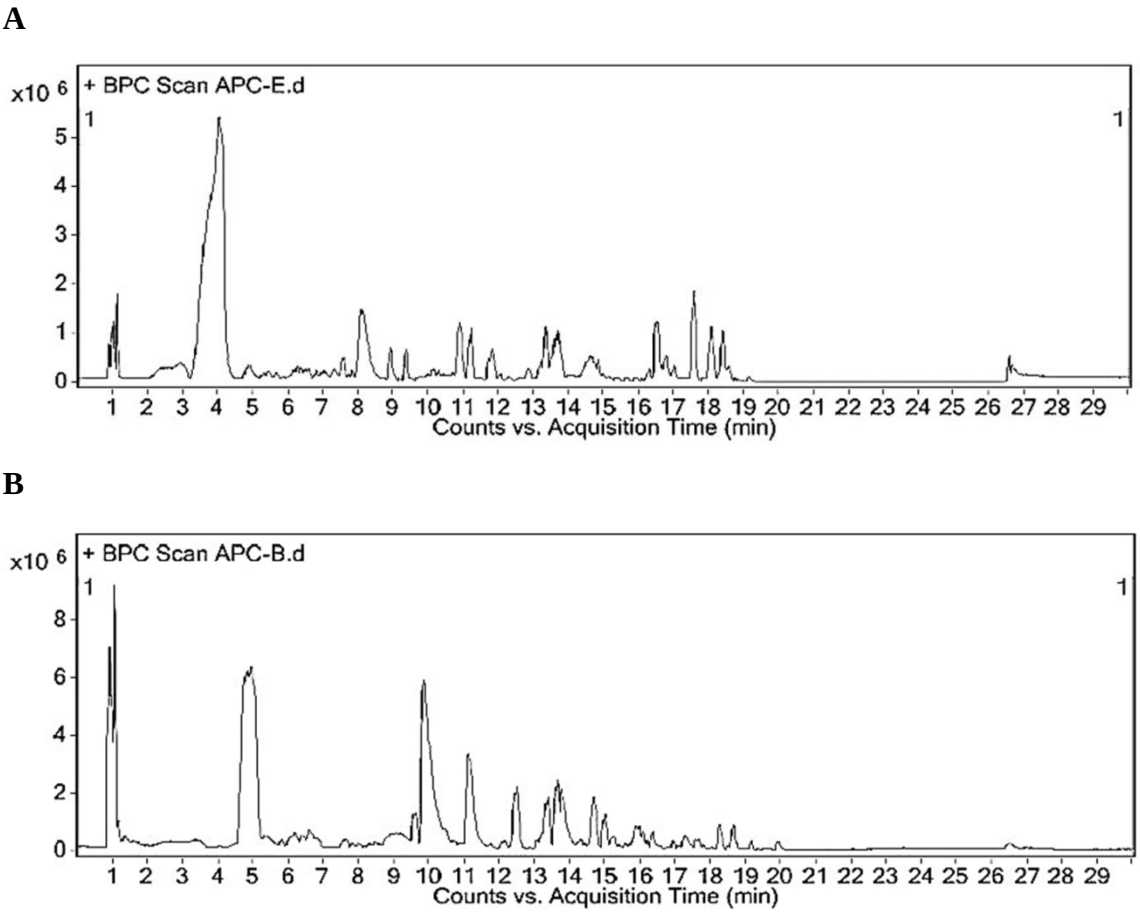


Figure 5.8.: (A) LC-MS chromatogram of ethyl-acetate fraction of APTE and **(B)** n-butanolic fraction of APTE.

LCMS analysis for the identification of the constituent compounds. The detailed list of compounds identified from AP(E) and AP(B) fractions are appended in appendix. The molecular formula and structures of seven anti-cancer compounds from AP(E) and five from AP(B) fractions were presented in **table 5.1. and 5.2.** respectively.

Table 5.1.: List of the anticancer compounds present in AP(E) fraction of APTE

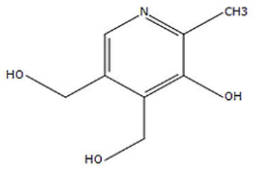
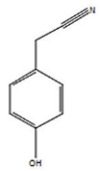
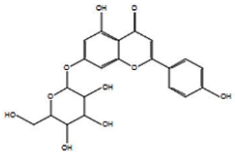
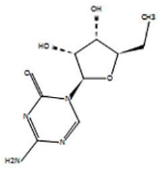
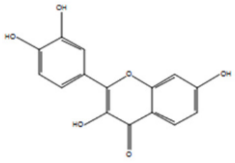
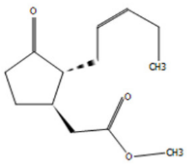
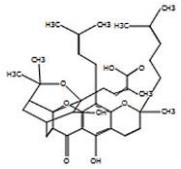
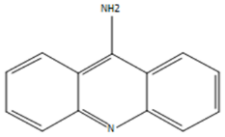
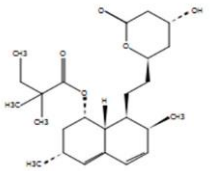
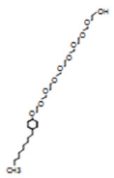
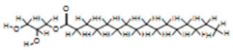
No	t_R (min)	Molecular Formula	Compound name	Structure	References
1	1.033	$C_8H_{11}NO_3$	Pyridoxine (Vitamin B6)		[17]
2	1.038	C_8H_7NO	4-Hydroxybenzyl cyanide		[18]
3	6.274	$C_{21}H_{20}O_{10}$	Cosmosiin		[19]
4	6.372	$C_8H_{12}N_4O_5$	Azacitidine		[20]
5	6.828	$C_{15}H_{10}O_6$	Fisetin		[21]
6	7.348	$C_{13}H_{20}O_3$	Methyl jasmonate		[22]
7	18.026	$C_{38}H_{46}O_8$	Dihydrogambogic acid		[23]

Table 5.2.: List of the anticancer compounds present in AP(B) fraction of APTE

No	t_R (min)	Molecular Formula	Compound name	Structure	References
1	11.143	$C_{13}H_{10}N_2$	9-Aminoacridine		[24]
2	13.803	$C_{16}H_{35}NO_2$	C16-Sphinganine		[25]
3	17.289	$C_{25}H_{38}O_5$	Simvastatin		[26]
4	17.292	$C_{33}H_{60}O_{10}$	Nanoxynol		[27]
5	18.227	$C_{19}H_{38}O_4$	1-Monopalmitin		[28]

Discussion

Amorphophallus paeoniifolius is a widely distributed medicinally important plant and its tuber is consumed as a vegetable in India. There are reports on the traditional use of this plant as medicine [15,29,30]. There are reports that demonstrated its anti-inflammatory, hepatoprotective, anthelmintic and analgesic properties [31-36]. To understand the mechanism of its anti-cancer activity, a hydroethanolic extract of the tuber of this plant was prepared by cold extraction method to ensure maximum extraction of bioactive compounds [37]. The extract induced near similar cytotoxicity to both MCF-7, an estrogen receptor-positive and MDA-MB-231, a triple-negative breast cancer cell line without affecting normal cells **(Fig 3.2.C & 3.5.B)**. This suggested that

the APTE-mediated cell death might be an estrogen signaling-independent event. Our observation was comparable to cytotoxicity of a methanolic extract of the same plant part against MCF-7 cells [30]. A different species, *Amorphophallus campanulatus* tuber was reported to induce cytotoxicity and apoptosis in colon and hepatic cancer cell lines [38,39]. Tuber from other plants e.g. *Cyperus rotundus* also reported being cytotoxic against murine lymphoblastic leukemia cells in a concentration range of 50-800 µg/ml [40]. *Ipomoea batatas* L. Lam, Taiwanese purple-fleshed sweet potato was reported for its cytotoxic and apoptosis-inducing activity against breast and gastric cancer cell lines in higher concentration range of 1-12 mg/ml [41]. Juice of another common Indian vegetable, *Momordica charantia* also induced apoptosis in MCF-7 and MDA-MB-231 cells as shown by PARP cleavage and Caspase 3 and 7 activity [42].

An anti-metastatic formulation will be a potent weapon in breast cancer management as there are no anti-metastatic drugs available in the market. No literature is available on the anti-metastatic effect of *A. paeoniifolius*. Migration, adhesion and invasion are three major hallmarks of the metastatic event. To understand the role of APTE on these properties *in vitro*, two sub-lethal concentrations of the extract were used which ascertained that the effect was exclusively due to the potency of the extract and not cell death. Similar concentrations (10 µg/ml, 25 µg/ml or 50 µg/ml) of the methanolic extracts of seed and leaf of *Eriobotrya japonica* showed dose-dependent inhibition in migration, adhesion and invasion in MDA-MB-231 cells [43]. Extracts prepared from various other plants demonstrated *in vitro* anti-metastatic activity in a wide range of effective concentrations which varied from 0.05 µg/ml to 1288 µg/ml [44,45]. Migration of breast cancer cells from the primary tumor to distant sites like bone, lungs, liver and brain is an integral step in metastatic initiation [46,47]. Wound healing assay suggested significant dose-dependent inhibition of migration by APTE in both the cells. Cell adhesion molecules responsible for cell to cell interaction for adhesion to each other are essential in the cascade of metastasis [48]. APTE treatment showed significant reduction in adhesion of the highly metastatic MDA-MB-231 cells to collagen IV coated suggesting possible its possible implication as an anti-metastatic compound. The final stages of cancer metastasis are the invasion of the cancer cells to extracellular matrix of distant tissues and grow the secondary tumors there [49,50]. The observation was further supported by inhibition of invasion in MDA-MB-231 cells by APTE. In contrast, effect of APTE on inhibition of adhesion and invasion of low metastatic MCF-

7 cells was nominal. Greater inhibition of *in vitro* metastatic activity in highly aggressive MDA-MB-231 cells suggested that this tuber extract could be important as a source of anti-metastatic compound.

Induction of apoptosis is a possible mode of cell death in cancer cells [51-53]. When cells undergo apoptosis the DNA in the cells starts degrading and a ladder-like smear of fragmented DNA is seen in the agarose gel [54]. Genomic DNA isolated from the cells after the treatment of APTE (125 µg/ml) in 48 hr showed DNA degradation in both cells and indicated the efficacy of APTE to induce apoptosis in both the cells. Treatment of APTE induced significant apoptosis in both MCF-7 and MDA-MB-231 cells. A dose-dependent increase of pro-apoptotic Bax and inhibition of anti-apoptotic Bcl-2 indicated induction of apoptosis by the extract [55,56]. Bax is a key player for apoptosis via mitochondrial stress and enhances membrane-permeability. This results in the release of cytochrome c from mitochondria and initiation of caspase mediated pathway for apoptosis. There are reports that suggest p53-mediated induction of Bax [56]. However, APTE treatment did not induce p53 expression suggesting Bax activation, in this case, might be independent of p53 [56,57]. The expression of caspase-7 is a major contributor to the execution of apoptosis. It is cleaved by different enzymes whose expressions are upregulated during apoptosis. The activated caspase-7 is responsible for cleavage of many substrates, including PARP. APTE successfully induced caspase-7 activation leading to cleavage of PARP in experimental cells [58,59]. These observations suggest that APTE induces apoptosis in both MCF-7 and MDA-MB-231 cells possibly by a caspase-7 dependent [60].

Identifying constituent compounds of an active extract is important to correlate association with anticancer effect. HR-LCMS analysis of the active ethyl acetate fraction of the extract showed the presence of seven tentative compounds with reported anticancer activities. Among these compounds, Pyridoxine (Vitamin B6), in combination with other vitamins and zinc, showed anticancer activity against bladder cancer [61]. The active form, pyridoxine 5′-phosphate, targeted pyridoxine 5′-phosphate oxidase and down-regulated its expression in ovarian cancer cells leading to reduced proliferation [17]. 4-hydroxybenzyl isothiocyanate, a derivative of 4-hydroxybenzyl cyanide, acted as anti-cancer agent against human neuroblastoma (SH-SY5Y) and glioblastoma (U87MG) cells [18]. The flavonoid Cosmosiin induced apoptosis in

stomach cancer cells and reduced risk of breast cancer [19]. The beneficial effect of Azacitidine, a pyrimidine nucleoside analog, was observed in patients with myelodysplastic syndrome and acute leukemic [20,62]. Fisetin, a well-known flavonoid, showed anti-cancer and anti-metastatic activity on various cancer cell lines like HL-60, SK-HEP-1, AsPC-1, SiHa and CaSki, HT-29, MCF-7[21,63-67]. Plant stress hormone methyl jasmonate also demonstrated anti-oxidant activity and inhibited metastasis and angiogenesis of cancer cells [22]. Finally, dihydrogambogic acid showed potent anticancer activity against cell lines like MCF-7, HT-29, T47D [23,68].

Five tentative compounds with known anti-cancer activity were found in butanolic fraction. Among these compounds, 9-Aminoacridine was reported to inhibit NF-kB phosphorylation and stimulate p53 signaling leading to cancer cell death [24]. C16-Sphinganine identified in *Brucea javanica* extract induced apoptosis in HCT-116 colon cancer cells [25]. Simvastatin, another compound identified in the butanolic fraction, was derived synthetically from the fermentation of *Aspergillus terreus*. It demonstrated anticancer activity in prostate (LNCaP and PC3) and colon cancer cells (COLO 205 and HCT 116) as well as in xenograft models [26,69]. Nonoxynol-9, a surfactant microbicide, induced apoptosis in endometrial explant model by caspase-dependent and independent pathways [27]. 1-Monopalmitin demonstrated a cytotoxic effect against liver cancer cell lines and induced apoptosis by NF-kB and Bcl2 mediated pathway [70]. We postulate that the combination of the compounds identified by LCMS might give a synergistic effect in killing the MCF-7 and MDA-MB-231 cells. However, there are different factors like structures of the compounds, their interaction, bioavailability of the compounds in the system, gene expressions resulted from their combination etc which affect the synergistic effect of any two or more compounds. This portion is been included in the discussion portion of the revised thesis in Chapter 5 in Page no 150. Overall from this study it is evident that APTE is a potent reservoir of well-characterized anti-cancer compounds. To our knowledge, this report describes the mechanism of anti-cancer activity *A. paeoniifolius* for the first time. Hence, this study potentially contributes to the growing prospect of medicinal plant-derived therapies for cancer.

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