RESEARCH ARTICLE

In vitro cytotoxic activity of Zaleya Pentandra L. Extracts against the breast cancer adenocarcinoma cell line MCF-7

Tahira Aziz Mughal[1], Fazilat Aslam[2], Zubaida Yousaf[3], Numrah Nisar[4], Ping Chung Leung[5]

Abstract

Objective: To evaluate the cytotoxicity of crude aqueous and ethanol extracts of Zaleya pentandra against oestrogen receptor-positive breast cancer cell line Michigan Cancer Foundation-7.

Method: The study was conducted at the Institute of Chinese Medicine, the Chinese University of Hong Kong, Hong Kong, from March to September 2017, and comprised Zaleya pentandra herbaceous perennial plant collected from Pakistani cities of Shakargarh, Lahore and Sialkot. Both aqueous and ethanol extracts were prepared in solvents following Soxhlet extraction technique. Rate of reduction in viability of cancer cell line was studied through MTT (dye compound 3-(4,5-*Dimethylthiazol*-2-yl)-2,5-*diphenyltetrazolium bromide*) assay on the basis of time of incubation and the dose of extract. Analysis was performed using High Performance Liquid Chromatography with Diode Array Detector in order to found the compounds responsible for the death of cancer cells.

Results: Cell viability was observed to be dose-dependent (range: 16.7-37.4%) for aqueous extracts. Minimum inhibition concentration was 16.65% at 200µg/ml after 24 hours of incubation, whereas maximum inhibition was 37.39% at 3200µg/ml. Ethanol extracts showed less inhibition, with maximum inhibition being 25.29% at 1000µg/ml and minimum 13.57% at 62.5µg/ml. Certain polar compounds, like Hydroxytrosol and Tyrosol, could be obtained from the aqueous extracts only.

Conclusion: Zaleya pentandra aqueous extract was found to have potential benefit towards cytotoxicity of breast cancer cells.

Keywords: HPLC-DAD, Zaleya pentandra, ZP, Ethanol extracts, Cytotoxicity. (JPMA 70: 35; 2020).
https://doi.org/10.5455/JPMA.299690

Introduction

Cancer, a disease as old as the humankind, despite all the advancements in medical sciences, is still a major health issue worldwide.[1] There have been 28 types of cancers reported in 184 countries of the world. In 2008, 12.7 million new cancer cases and 7.6 million cancer-related deaths took place, while almost 14.1 million new cancer cases and 8.2 million cancer-related deaths were recorded in 2012, indicating an alarming increase.[2] It was also estimated that the number of new cases would rise to 19.3 million per year by 2025. More than half of all cancers (56.8%) and cancer-related deaths (64.9%) in 2012 occurred in the less developed world.[2]

[1,4]Department of Environmental Science, Lahore College for Women University, Lahore, Pakistan; [2,3]Department of Botany, Lahore College for Women University, Lahore, Pakistan; [5]Institute of Chines Medicine, The Chines University of Hong Kong, Hong Kong.
Correspondence: Tahira Aziz Mughal. e-mail: drtahiramughal@gmail.com

Breast cancer is the leading cause of cancer deaths among females, and it is the most frequently diagnosed cancer, accounting for 23% of the total and 14% of related deaths.[3] In recent years it has become clear that breast cancer collectively consists of many molecularly-distinct tumours arising from the epithelial cells of the breast and does not represent a single disease.[4] In cancer research done using in vitro models, cell lines seem to be a key component for the molecular diagnosis in breast cancer cases as they can be widely used in numerous features of laboratory research.[5] Among all the breast cancer cell lines, Michigan Cancer Foundation-7 (MCF-7) cells represent an important candidate as they are used universally in research for oestrogen receptor (ER)-positive breast cancer cell experiments and many of its sub-clones with varying nuclear receptor expression levels.[6]

MCF-7 cell line has been propagated for many years by

multiple groups and is the most regularly used line in laboratory research worldwide as it is usually known to have low metastatic potential, and is a poorly-aggressive and non-invasive cell line.[7-9]

Clonal variations have been found in MCF-7 cells as extensive aneuploidy with important variations in chromosome numbers ranging 60-140 having been observed. Additional cytogenetic variances have also been reported about the presence or absence of specific marker chromosomes.[10] Literature suggests a raised level of genetic instability in MCF-7 cells. Due to different culture conditions, karyotype differences could reflect changes in selective pressure. Besides, MCF-7 cells comprise a division of stem cells able to create clonal variability, and, as a model for breast tumour heterogeneity, this was proposed as an explanation for the heterogeneity of this cell line. Both the genomic and ribonucleic acid (RNA) expression levels of different MCF-7 variants undergo divergence.[10]

MCF-7 cells have been used universally in research for oestrogen receptor (ER)-positive breast cancer cells. MCF-7 cells are compatible for anti-hormone therapy resistance studies since they are easily cultured and, when treated with such targeted-therapy, they hold ER expression. Populations of MCF-7 cells modified to various anti-hormone environments have been produced in order to explore the properties of acquired anti-hormone resistant breast cancer cells. The only breast cancer cell line which has produced more data ever of practical knowledge for patient care than any other is the MCF-7 cell line.[6]

Traditionally, herbal drugs have played a vital role in the cure of different diseases. In recent years, plant-based compounds have been preferred. Quinoline isolated from Quinine plant has been used in many synthetic medicines.[11]

Zaleya Pentandra (Linn.) (AP) belongs to the genus Zaleya (Family: Aiozaceae). It is a prostrate and branched herb species found in Pakistan, especially in southern Punjab, Azad Kashmir, Cholistan and Nara deserts of Balochistan.[12,13] Six Zaleya species are reportedly used in traditional medicines to treat and manage cancer, bronchial disease, fever, constipation, urinary tract infection as well as kidney and bladder stones etc.[14]

ZP has anti-ulcerogenic activity through some unknown mechanism and it has been used in the area by traditional healers for a vast variety of ailments. Besides, it is also used as fodder for cattle in some desert areas.[15-17] The plant reportedly contains antioxidant activity as well.[18]

Pentandraon, a novel compound which is chemically a steroidal hormone, was isolated from nethanolic extract of ZP in the form of amorphous solid.[12] Another steroidal compound, named pentandraol, was isolated as amorphous solid from the methanolic extract of ZP which is probably responsible for the antibacterial activity against Bacillus (B.) spizizenii and Staphylococcus (S.) aureus bacterial strains, and the methanolic extract also showed antioxidant activity.[13] Another compound , pentandradiol, was isolated from the methanolic extract of ZP. The dichloromethane extract of ZP showed significant anti-diabetic activity against alpha (α)-glucosidase.[19]

The current study was planned to evaluate the cytotoxicity of crude aqueous and ethanol extracts of ZP against ER-positive breast cancer cell line Michigan Cancer Foundation-7 (MCF-7).

Materials and Methods

The study was conducted at the Institute of Chinese Medicine, the Chinese University of Hong Kong, Hong Kong, from March to September 2017, and comprised ZP herbaceous perennial plant collected from Pakistani cities of Shakargarh, Lahore and Sialkot.

Fresh plant material, including root, stem and leaves, was processed by washing with distilled water to remove the foreign matter, like dust, dung, insects. They were then shade-dried and crushed with pestle and mortar into fine powder particles and stored for further experiments. The plant specimens were identified by Prof. Dr. Zaheeruddin Khan, a well-known taxonomist at the Government College University, Lahore, (www.gcu.edu.pk) and voucher specimen was deposited (PM # 667) in Prem Madan Herbarium, Lahore College for Women University, Lahore (www.lcwu.edu.pk). Water and ethanol were used as solvents; one is polar and the other non-polar. ZP extraction were carried out by Soxhlet extraction method.[20] Ethanol and water extraction were carried out at 60°C and 100°C respectively. Ethanol was evaporated under vacuum by rotary evaporator to yield crude ethanol extract. The water extract was first centrifuged and then stored in Freeze-dried mode. All solvents used were of analytical grade (Merck).

ZP aqueous extract 32mg/ml was used to dissolve in the full Roswell Park Memorial Institute (RPMI) medium 1640 (Invitrogen GIBCO, NY, USA) containing 10% Foetal Bovine serum (FBS; Invitrogen GIBCO) and 1% penicillin, 1% streptomycin (PS; Invitrogen GIBCO) to prepare the stock solution while the highest concentration was 3.2mg/ml. The ethanol extract 200mg/ml was dissolved in dimethyl sulfoxide (DMSO; Sigma) first as stock solution and then in full RPMI medium to get the highest concentration 1mg/ml. The extracts were completely homogenised using ultra homogeniser (ultrasonic cleaner Branson 5510-sigma Aldrich). The 0.22μm syringe filter was used to sterilise and filter the aqueous and ethanol stock solutions. Further dilutions were made with the full culture medium RPMI to the defined concentrations.[21]

Human breast cancer cell line MCF-7(American Type Culture Collection [ATCC], MD, USA). These cells originated from a metastatic adenocarcinoma which expressed ER. The cell lines were maintained in a humidified incubator at 37°C in 5% carbon dioxide (CO2) atmosphere.

RPMI medium 1640, supplemented with 10% FBS (Invitrogen GIBCO), 100 units / ml penicillin and 100μg/ml streptomycin (Invitrogen GIBCO). During MCF-7 harvesting, trypsin was used as the detaching agent for cells. Culture flasks containing RPMI medium with supplements were then transferred into new culture flasks for further growth and activation of cell lines. The cell lines were transferred into new culture flasks more than 4 times so that all the cells could get completely activated.

Haemocytometer was used to count the harvested cells while the trypan blue exclusion test was used to determine cell viability. The 96-well flat-bottom Costar culture plates (Corning Inc., MA, USA) were used to seed the MCF-7 cells while keeping cell density $5x10^4$ cells seeded per well in 100μl of culture medium RPMI 1640 supplemented with 10% FBS, 1% penicillin and 1% streptomycin. The defined concentrations of the aqueous and ethanol ZP extracts were prepared in the culture medium containing MCF-7 cells, and 100μl of solution was added to each well. Control wells were added with 100μl of plain culture medium with MCF-7 cells. And 96 well plates containing cultured MCF-7 cells along with the ZP were then incubated for a defined time of 16 hours, 24 hours and 48 hours. ZP extract treated MCF-7 cells. Proliferative response and cell death were determined using MTT cytotoxicity assay and cell viability assay. MTT is the abbreviation for the dye compound 3-(4,5-*Dimethylthiazol*-2-yl)-2,5-*diphenyltetrazolium bromide*. This dye is helpful measuring the functionality (Cytotoxicity) of animal and human cells by calculating the activity of enzymes that reduce MTT. That is why it is called MTT assay.[21] After incubation of MCF-7 cells with the ZP extract for 16, 24 and 48 hours in 96-well plates, they were taken out of the incubator and 30μl of 5mg/ml MTT in phosphate-buffered saline (PBS; Invitrogen GIBCO) was added to each well and the plates were incubated again at 37°C for 3-4 hours. They were then taken out and the entire liquid medium was pipetted out of the wells. One hundred microliter of DMSO (Sigma) was then added to each well. Absorbance of the dissolved solution was detected at 540nm by a Benchmark microtiter plate reader (Bio-Rad Laboratories, CA, USA). The absorbance of untreated cultured cells was considered 100%.[22]

$$\text{Proliferation rate} = \frac{At - Ab}{Ac - Ab}$$

$$\text{Percentage viability} = \frac{At - Ab \times 100}{Ac - Ab}$$

$$\text{Percentage inhibition} = \frac{100 - At - Ab \times 100}{Ac - Ab}$$

Where,
At=Absorbance value of tested plant extract
Ab=Absorbance value of blank
Ac=Absorbance value of negative control (untreated cells)

To evaluate the effect of the ZP extract on cell viability, cells were exposed to the ZP extract at concentrations producing 50% growth inhibition (IC50) for the tumour cell lines, as determined by MTT assay, for 24 and 48 h. The viable cells were counted by trypan blue exclusion test. The percentage of dead cells was calculated from the ratio of dead cells to total number of cells x 100%.[21]

Both the aqueous and ethanolic extracts were subjected to HPLC analysis (Waters 600 Controller System) with the separation achieved at thermo-scientific NX 5μM C18 column (250×4.6 mm) at 25°C. The detector photodiode detector (Waters 432 Controller System) was set at the wavelength of 235nm. Samples were sonicated briefly and passed through 0.45μm membrane filter

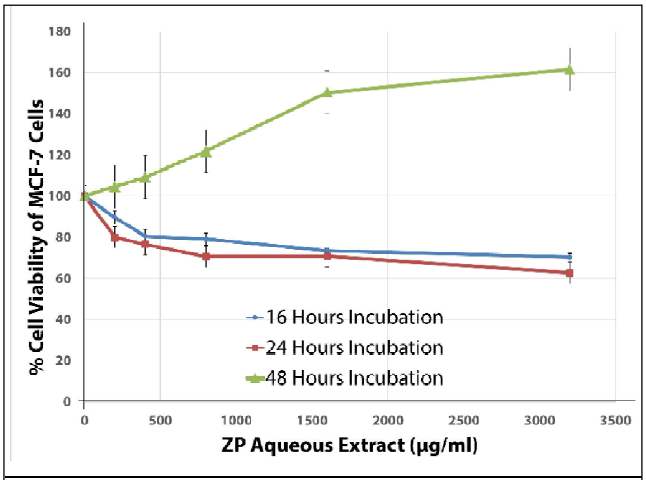

Figure-1: Effect of Aqueous Extract of Zaleya Pentandra (ZP) on % Cell Viability of Michigan Cancer Foundation-7 (MCF-7) cell line after 16, 24 and 48 Hours Incubation Treatment during MTT assay. All the experiments were performed independently in triplicate manner for each Incubation Period.
n: 3 SD±, (p < 0.005)

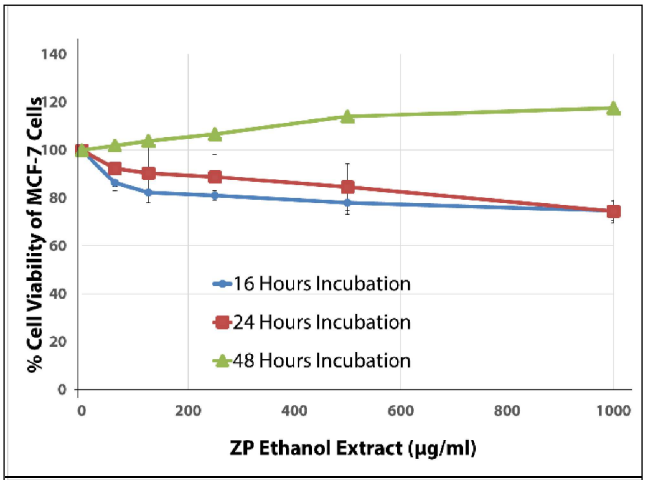

Figure-2: Effect of Ethanol Extract of Zaleya pentandra (ZP) on % Cell Viability of Michigan Cancer Foundation-7 (MCF-7) cell line after 16, 24 and 48 Hours Incubation Treatment during MTT assay. All the experiments were performed independently in triplicate manner for each Incubation Period.
n: 3 SD±, (p < 0.005)

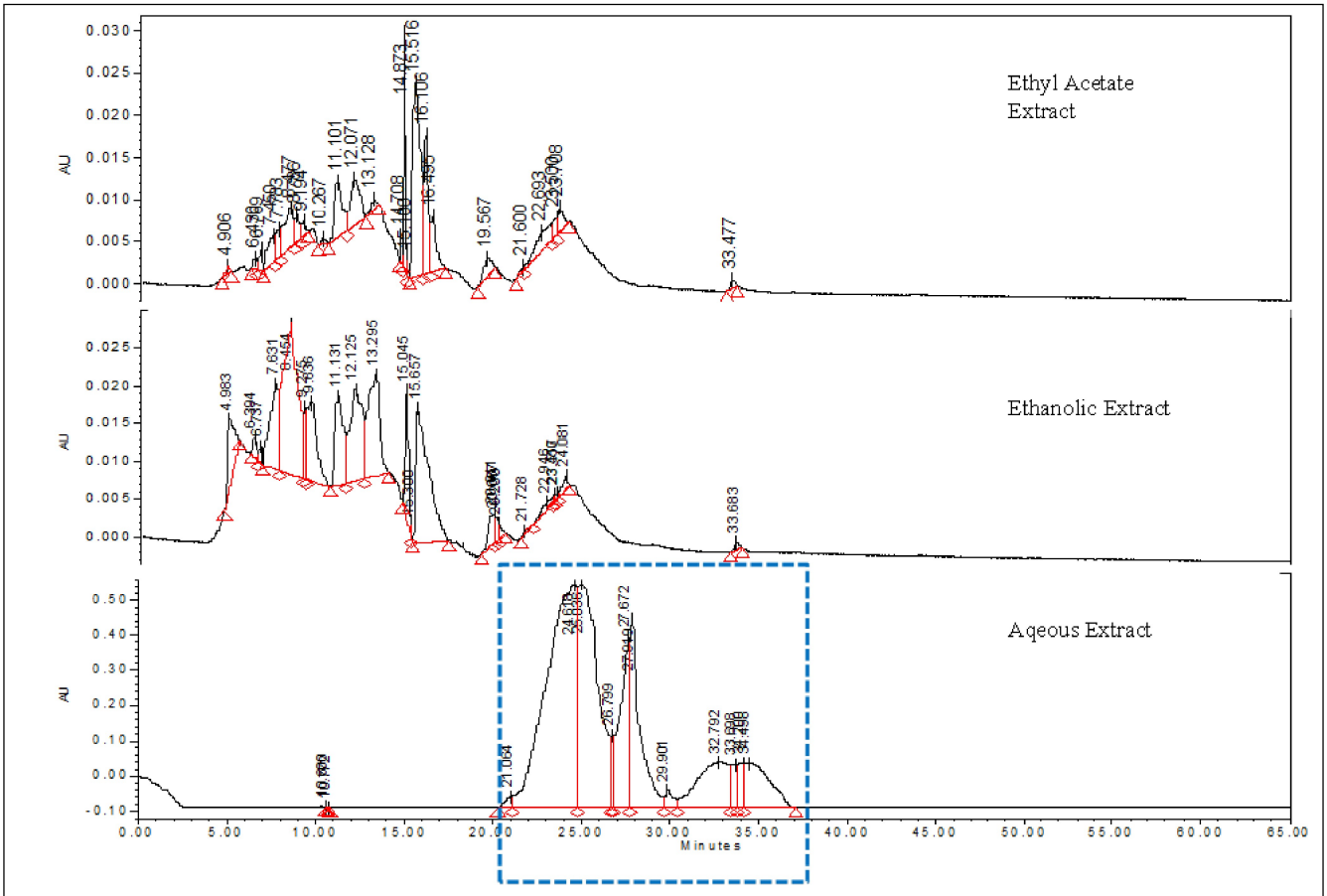

Figure-3: High Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD) analysis of three extracts Zaleya Pentandra (ZP). As can be seen that blue highlighted portion has been extracted more in aqueous extracts which is responsible for the increased cytotoxicity of Michigan Cancer Foundation-7 (MCF-7) Breast Cancer cell line.

paper (Whattman, GE Healthcare) before analysis. Solvent phase was methanol (solvent A), and 0.1% formic acid in HPLC grade water (solvent B). Gradient programme commenced at 5% of A and 95% of B at 0 min reaching to linear gradient of 100% A and 0% B over 65 min and held at for 10 min in that composition at 1ml/min flow rate. The nature of the compounds was determined by comparing it with the literature data.[23,24,25] Further confirmation and concentration was achieved through Vicenin-2 as reference standard, due to its availability, following the protocol as suggested by Gouvea et al., 2017. Vicenin-2 is a flavonoid exhibit anti-inflammatory and anti-cancer activity and found in hydro alcoholic plant extracts.[26] The reference compound was dissolved in water and ethanol in similar manner as the samples.

The experiments were performed using complete randomised design in triplicate manner with six replicates for each tested dilution / concentration of all the crude extracts. Data was expressed as mean% MTT absorbance (ratio of absorbance in extract-treated well to that of control well x 100%) ± standard deviation (SD) of three independent experiments with six wells each for in vitro studies. Data was entered in the form of spreadsheet in Microsoft excel and statistical analysis was done using one-way analysis of variance (ANOVA) in SPSS 25.0. $P<0.05$) was considered statistically significant.

Results

All the treatments with ethanol extract for 16 hours and 24 hours showed ER-positive MCF-7 breast cancer cells inhibition and the results were statistically significant ($p<0.05$) for 250, 500 and 1000µg/ml. The treatment at 48 hours with ethanol extract did not show the significant inhibition for cancer cells ($p>0.05$).

All the treatments with ZP aqueous extracts showed apoptosis of cancer cells. The 200 and 400µg/ml aqueous extract treatment showed significant inhibition ($p<0.05$), while the 800, 1600 and 3200µg/ml aqueous extract treatment showed significant inhibition of ER-positive MCF-7 breast cancer cells ($p<0.005$) for 16 and 24 hours treatment. At 48 hours, no significant inhibition was observed ($p>0.05$) (Figures 1-2).

HPLC-DAD analysis revealed certain important polar and partially polar compounds from the extracts (Table). Chromatograms showed certain compounds from aqueous extracts with Retention Time 24, 27 and 35 minutes (Figure 3).

Table: Identification of different peaks through High Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD) analysis on the basis of internal standard and HPLC library search and their possible enumeration on the basis of earlier literature.

Sr. NO.	Name of Compound	Retention time (min)	HPLC-DAD (nm)
1	Kaempferol 3- (2',3'dyacetylrhamnoside)- 7'-rhamnoside	5	280
2	Unknown polar compounds	6	280
3	Unknown polar compounds	7	280
4	Hydroxytrosol	9	280
5	Internal standard	11	280
6	Tyrosol	12	280
7	Vanillic acid	13	280
8	p-coumaric acid	15	280
9	Ferulic acid	15.6	280, 324
10	Hydroxytyrosol acetate	20	280
11	Dialdehydic form of oleuropein Aglycone (Decardoxymethylated)	23	280
12	Acetoside	24	280
13	Pinoresinol	28	
14	Kampferol-7-0 glucoside	33	280

Discussion

Extracts of ZP active in crude form were found to have a dose-dependent efficacy just like some studies have found in the case of *Morinda lucida and Catharanthus roseus*, reporting that activity increased with increase in concentration.[27-30]

The aqueous extract of ZP proved to be highly effective against apoptotic induction of cell death compared to the ethanol extract. Results revealed that higher the concentration, maximum would be the apoptotic cell cycle arrest and cell death. In case of aqueous extract, the maximum inhibition was observed in the highest concentration of 3200µg/ml treatment with ($p<0.005$), while in ethanol extract, maximum inhibition of ER-positive MCF-7 breast cancer cells was observed in the highest concentration of 1000µg/ml treatment. Maximum activity in aqueous fraction proved that compounds responsible for activity were polar in nature. Increase in activity while moving towards polar solvent indicated the presence of compounds in different fractions, but their quantity might be increased in aqueous fraction due to ability of water to dissolve more compounds than the other solvents. Water decoction is more effective than other non-polar extracts.[31] The polarity trend was also seen in fractions of *Aesculus indica*

and *Aspidosperma tomentosum*.[32]

ZP is herbaceous perennial plant that has not been fully explored in laboratory to know its chemical profile and bioactive ingredients. The results of the current study, among other things, provide evidence of time-dependent efficacy of ZP extracts. Vicenin-2 is a flavonoid exhibit anti-inflammatory and anti-cancer activity and found in hydro alcoholic plant extracts. The presence of vicenin-2 in both extracts as evident from the HPLC analysis, revealed the secret behind the anti-cancer property of ZP extracts.[26]

ER-positive MCF-7 cells proliferation of cancer cells might be due to the presence of steroidal hormone-like compounds, such as pentandraon, pentandraol and pentandradiol, in the crude extract of ZP as has been reported in literature.[12,13,19]

Future studies on animal models are recommended in order to better understand the mechanism of ZP action and to move towards low-cost anti-cancer herbal drugs.

Conclusion

Zaleya pentandra aqueous extract was found to have potential benefit towards cytotoxicity of breast cancer cells.

Disclaimer: The text is part of a PhD thesis.

Conflict of Interest: None.

Source of Funding: The Higher Education Commission of Pakistan.

References

1. Winans B, Humble MC, Lawrence BP. Environmental toxicants and the developing immune system: a missing link in the global battle against infectious disease. Reprod Toxicol 2011; 31: 327-36.
2. WHO. Latest world cancer statistics Global cancer burden rises to 14.1 million new cases in 2012: Marked increase in breast cancers must be addressed. France, International Agency for Cancer Research; 2013.
3. Gunduz M, Gunduz E. Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways. [Online] [Cited 2018 May 18]. Avaialble from: URL: https://www.intechopen.com/books/breast-cancer-carcinogenesis-cell-growth-and-signalling-pathways
4. Done S. Breast Cancer-Recent Advances in Biology, Imaging and Therapeutics. [Online] [Cited 2018 May 18]. Avaialble from: URL: https://www.intechopen.com/books/breast-cancer-recent-advances-in-biology-imaging-and-therapeutics
5. Burdall S, Hanby A, Lansdown MR, Speirs V. Breast cancer cell lines: friend or foe? Breast Cancer Res 2003; 5: 89-95
6. Sweeney EE, Mcdaniel RE, Maximov PY, Fan P, Craig V. Models and Mechanisms of Acquired Antihormone Resistance in Breast Cancer: Significant Clinical Progress despite Limitations. Horm Mol Biol Clin Investig 2013; 9: 143163
7. Baguley BC, Leung E. Heterogeneity of Phenotype in Breast Cancer Cell Lines. in Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways (Gunduz M, Gunduz E (eds.). [Online] [Cited 2018 May 18]. Avaialble from: URL: https://www.intechopen.com/books/breast-cancer-carcinogenesis-cell-growth-and-signalling-pathways/heterogeneity-of-phenotype-in-breast-cancer-cell-lines
8. Shirazi FH. Remarks in Successful Cellular Investigations for Fighting Breast Cancer Using Novel Synthetic Compounds. [Online] [Cited 2018 May 18]. Avaialble from: URL: https://www.intechopen.com/books/breast-cancer-focusing-tumor-microenvironment-stem-cells-and-metastasis/remarks-in-successful-cellular-investigations-for-fighting-breast-cancer-using-novel-synthetic-compo
9. Gest C, Joimel U, Huang L, Pritchard LL, Petit A, Dulong C, et al. Rac3 induces a molecular pathway triggering breast cancer cell aggressiveness: differences in MDA-MB-231 and MCF-7 breast cancer cell lines. BMC Cancer. 2013; 13: 63
10. Nugoli M, Chuchana P, Vendrell J, Orsetti B, Ursule L, Nguyen C, et al. Genetic variability in MCF-7 sublines: evidence of rapid genomic and RNA expression profile modifications. BMC Cancer 2003; 12: 1-12
11. Kokate C, Purohit A, Gokhale S. Pharmacognosy. India: Nirali Prakashan, 2007; pp. 635.
12. Samina A, Bashir AC, Muhammad U, Khurram A, Tasveer ZB. Isolation Of Pentandraon From Methanolic Extract Of Aerial Parts Of Zaleya pentandra. Int J Pharm 2013; 4: 21-3.
13. Afzal S, Chaudhry BA, Saeed J, Afzal K, Ahmed B, Qadir MI. Antibacterial And Antioxidant Activity Of methanolic extract Of ZaleyaPentandra. Acta PoloniaePharmaceutica Drug Res 2016; 73: 147- 51
14. Asaa MHHB, Murtaza G, Siraj S, Khan SA, Azhar S, Hussain MS, et al. Enlisting the scientifically unnoticed medicinal plants of Pakistan as a source of novel therapeutic agents showing anti-venom activity. Afr J Pharm Pharmacol 2011; 5: 2292-305.
15. Hameed M, Ashraf M, Al-Quriany F, Nawaz T, Sajid M, Ahmad A, et al. Medicinal flora of the cholistan desert. A review. Pak J Botany 2011; 43: 39-50.
16. Mahmood A, Malik RN, Shinwari ZK, Mahmood A. Ethnobotanical survey of plants from Nelum, Azad Jammu and Kashmir, Pakistan. Pak J Botany 2011; 43: 105-10.
17. Akhtar AH, Ahmad KU. Anti-ulcerogenic evaluation of the methanolic extracts of some indigenous medicinal plants of Pakistan in aspirin-ulcerated rats. J Ethnopharmacol 1995; 46: 1-6.
18. Qasim M, Gulzar S, Khan MA, Halophytes as medicinal plants. NAM Meeting in Denizli, Turkey. 2011;
19. Afzal S, Nasir B, Hanif M, Afzal K, Abbas G, Saeed J. Extraction and Antidiabetic Evaluation of Zaleyapentandra. Latin Am J Pharm 2017; 36: 1563-7
20. Bruce E, RichterBAJ, John L, Nathan L. Accelerated Solvent Extraction:? A Technique for Sample Preparation, Anal Chem 1996; 68: 1033-9
21. Ho CY, Kim CF, Leung KN, Fung KP, Tse TF, Chan H, et al. Differential anti-tumor activity of coriolus versicolor (Yunzhi) extract through p53- and/or Bcl-2-dependent apoptotic pathway in human breast cancer cells. Cancer Biol Ther 2005; 4: 638-44
22. Lau CB, Ho CY, Kim CF, Leung KN, Fung KP, Chow MS, et al. Cytotoxic activities of Coriolus versicolor (Yunzhi) extract on human leukemia and lymphoma cells by induction of apoptosis. Life Sci 2004; 75: 797-808.
23. NIST Chemistry webbook SRD 69. [Online] [Cited 2018 March 18]. Available from: URL: http://webbook.nist.gov/chemistry
24. Spectral Data base for Organic compounds. [Online] [Cited 2018 March 18]. Available from: URL: https://sdbs.db.aist.go.jp
25. Kohn LK, Pizão PE, Foglio MA, Antônio MA, Amaral MCE, Bittric V, et al. Antiproliferative activity of crude extract and fractions obtained from Aspidosperma tomentosum Mart. Rev Bras Pl Med Botucatu 2006; 8: 110-5.

26. Gouvea DR, Buqui GA, Lopes JLC, Diniz A, Lopes NP. An UPLC-MS/MS Method for determination of Vicenin-2 and Lychnopholic Acid in Rat Plasma and its application to a pharmacokinetic study. J Braz Chem Soc 2017; 28: 427-34
27. Jagetia GC, Rao SK. Evaluation of cytotoxic effects of dichloromethane extract of guduchi (TinosporacordifoliaMiers ex Hook F & THOMS) on cultured HeLa cells. eCAM 2006; 3: 267-72.
28. Aisha AFA, Sahib HB, Abu-Salah KM, Darwis Y, Abdul Majid AMS. Cytotoxic and antiangiogenic properties of the stem bark extract of Sandoricum koetjape. Int J Cancer Res 2009; 5: 105-14.
29. Rahman MA, Rana MS, Zaman MM, Uddin SA, Akhter R. Antioxidant, antibacterial and cytotoxic activity of the methanol extract of Urtica Crenulata. J Sci Res 2010; 2: 169-77.
30. Daniels AL, Van Slambrouck S, Lee RK, Arguello TS, Browning J, Pullin MJ, et al. Effects of extract from two native American plants on proliferation of human breast and Colon cancer cell lines in Vitro. Onco Rep 2006; 15: 1327-31
31. Kamuhabwa A, Nshimo C, de Witte P. Cytotoxicity of some medicinal plant extracts used in Tanzanian traditional medicine. J Ethnopharmacol 2000; 70: 143-9.
32. Kohn LK, Pizao PE, Foglio MA, Antonio MA, Amaral MCE, Bittric V, et al. Antiproliferative activity of crude extract and fractions obtained from Aspidosperma tomentosum Mart. Rev Bras Pl Med Botucatu 2006; 8: 110-5.