Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

ANTI-PROLIFERATIVE POTENTIAL OF *Carica papaya* LEAVES ON BREAST CANCER CELLS – MCF-7

Sasikala Mariyappan, Sivakumar Ramalingam, Lavanya Murugan, Renuka Saravanan*

Department of Chemistry and Biosciences, SASTRA Deemed to be University, Srinivasa Ramanujan Centre, Kumbakonam 612001, Tamil Nadu, India

Received – July 20, 2021; Revision – September 28, 2021; Accepted – October 12, 2021
Available Online – October 30, 2021

DOI: http://dx.doi.org/10.18006/2021.9(5).678.686

KEYWORDS

Breast cancer

MTT assay

DPPH assay

Caria papaya

MCF-7

ABSTRACT

The study's objective is to identify the phytoconstituents and determine the anti-cancer potential of *Carica papaya* leaves against the MCF 7 cell line. Chloroform, ethyl acetate, and methanol extracts of *C. papaya* leaves were prepared by cold maceration method and qualitative phytochemical analysis was performed. The anti-proliferative effect of these extracts was determined by 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and apoptotic assay by acridine orange/ethidium bromide staining method on MCF 7 cells. The effect of the extracts, with different concentrations, on DNA fragmentation, was also performed on MCF 7 cells. Qualitative analysis revealed the presence of alkaloids, flavonoids, terpenoids, steroids, saponins, tannins, glycosides, phenols, anthraquinones, proteins, and carbohydrates. Chloroform, methanol, and ethyl acetate extracts of *C. papaya* leaves were observed with potential DPPH free radical scavenging activity with 72%, 75%, and 78% respectively. Of these extracts, the chloroform extract (72%) was found to possess a more free radical scavenging effect against DPPH and also showed a dose-dependent effect, the maximum at 100µg/ml, on DNA fragmentation in MCF 7 cells. Further, chloroform extract showed a maximum anti-proliferative effect on MCF-7 cells with IC_{50} at 22±1.5µg/ml, whereas methanol and ethyl acetate extract at 30±0.5 µg/ml and 28±0.5 µg/ml respectively. Increased apoptosis in MCF 7 cells was observed with an increased concentration of chloroform extract of *C. papaya.* From the results of this study, it can be concluded that leaf extract of *C. papaya* found to possess an anti-proliferative effect and antioxidant potential and it could be due to the presence of rich secondary metabolites of the plant.

* Corresponding author

E-mail: renuka@src.sastra.edu (Renuka Saravanan)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Breast cancer is among the most common type of cancer in women around the globe (Yahyea et al., 2019). It is developed due to the inheritance of proto-oncogenes such as BRCA1, BRCA2 and by a genetic mutation. Most breast cancer is contributed by carcinoma, while sarcoma is rarely associated with this cancer (Feng et al., 2018). If the tumor is diagnosed early, it could be treated easily (Fadi & Ferguson, 2020). Although it is seen primarily in women, men can also get breast cancer (Farr et al., 2017). Chemotherapy and radiation therapy are the commonly used treatment to treat breast cancer. But, these treatments produce adverse side effects (Alam et al., 2013). The tumor development can be reversed or inhibited by synthetic drugs. Plant extract might play an essential role in preventing and curing breast cancer (Levitsky & Dembitsky, 2015). Medicinal plants are well recognized and have been used in the preparation of traditional medicines with fewer side effects to treat various diseases especially cancer (Richard et al., 2015). The active phytochemical constitutes of herbal plants are carotenoids, flavonoids, ligands, terpenoids, and sulfides stimulate glutathione-transferase that helps protect the cell and prevent the proliferation of the cell (Shareef et al., 2016).

Carica papaya belongs to the family of Caricaceae (Figure 1). It is diploid, dicotyledon, and polygamous plants. The papaya is consumed in the form of jam, juices, or dry fruit. The ripe fruit of *C. papaya* is rich in Vitamin C, A, and calcium. The different product of *C. papaya* is used to produce a variety of commercial products. Chymopapain and papain are made from latex, stem, leaves, and fruit. For many centuries, papaya leaves have been used as folk medicine. Recently researchers proved the wound healing, anti-inflammatory, anti-oxidant, immune-modulatory, and anti-tumor effect of this plant (Saurabh et al., 2016). Hence, the present study aims to analyze the phytoconstituents and to evaluate the anticancer potential of *C. papaya* leaves against the breast cancer cell line MCF 7 cells.

Figure 1 *Caria papaya* leaves

2 Materials and Methods

2.1 Collection of Plant material

The leaves of *C. papaya* were collected from Kumbakonam, Thanjavur District, Tamil Nadu, India. The leaves were shade-dried, powdered with an electrical blender, and stored in an airtight container till use.

2.2 Preparation of plant extract

About 25g of powder was immersed in 100 ml of chloroform, ethyl acetate, and methanol separately for 72 hours with occasional shaking at room temperature. The extract was collected after filtering using Whatman No1 filter paper and stored at 4°C till use. Then, the extracts were condensed and used for experiments (Kiruthika et al., 2020).

2.3 Qualitative analysis of phytochemical constituents

Qualitative analysis of phytochemical constituents was carried out by the methods of Harbone et al. (1998). The detail of the used method for qualitative analysis was given in table 1.

Table 1 Qualitative analysis of various phytochemical constituents

S.No	Phytochemical Constituents	Appeared colour/ Characteristics	Reference
1.	Alkaloids	Creamy white precipitate	Mustikasari & Ariyani, 2010
2.	Flavonoids	Intense red color	Chang et al., 2002
3.	Terpenoids	Reddish-brown color	Indumathi et al., 2014
4.	Steroids	Blue-green color	Silas et al., 2019
5.	Saponins	Appearance of froth	Kokate et al., 2001
6.	Tannins	Green-blue is formed	Schanderl,1970
7.	Glycosides	Formation of Green color	Kokate et al., 2001
8.	Phenols	Development of Blue color	McDonald et al., 2001
9.	Anthraquinones	Appearance of Rose pink color	Silas et al., 2019
10.	Proteins	Violet color is formed	Padmapriya & Maneemegalai, 2014
11.	Carbohydrates	Appearance of violet color	Padmapriya & Maneemegalai 2014

2.4 In-vitro anti-oxidant activity

The anti-oxidant assay was carried out by the method of Mensor et al. (2001). In these assays, DPPH was used as the standard free radical, when it reacts with anti-oxidants, gets reduced to DPPHH. Due to the reduction reaction, the absorbance of DPPHH decreases in comparison to DPPH. The anti-oxidant activity of

compounds or extracts is indicated by the degree of discoloration in terms of hydrogen donating ability. The anti-oxidant activity of the fraction was measured *in vitro* by 1, 1 diphenyl- 2-picrylhydrazyl (DPPH) assay (Mensor et al., 2001). In brief, 0.5 mM DPPH was prepared in 95% methanol, and 1 ml of this solution was added to the tubes containing 3ml of leaf extracts (chloroform, methanol, and ethyl acetate) at different concentrations. The contents of the tubes were mixed well, left at room temperature for 30 minutes, and the absorbance was measured at 515 nm in a spectrophotometer. The antioxidant activity was expressed as:

$$\% \text{ of disappearance} = \frac{\text{control} - \text{sample}}{\text{control}} \times 100 \quad \text{(Control - Sample)}$$

2.5 Cell line and culture conditions

The human breast cancer cells (MCF-7) were purchased from the National Center for Cell Sciences (NCCS), Pune, India. The cancer cells were maintained in MEM medium (MEM+FCS) supplemented with 2mM/L glutamine and balanced salt solution (BSS) adjusted to contain 1.5 g/L Na_2CO_3, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate,2 mM /L glutamine, 1.5 g/L glucose, 10 mM (4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid) (HEPES) and 10% fetal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100µg) were adjusted to 1mL/L. The cells were maintained at 37°C with 5% CO_2 in a humidified CO_2 incubator (Saravanan et al., 2017).

2.6 MTT assay

MTT [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay was used to evaluate the Inhibitory Concentration (IC_{50}) of the plant extracts (Joseph et al., 2020). The MEM (1×10^4cells/well) was used to grow the cells in a 96-well plate for 48 hours in 85% confluence. The medium was replaced with a fresh medium containing a serially diluted compound. The cells were then incubated for 48 hours. 100µL of the MTT [3-(4, 5-dimethylthiozol-2-yl)-3,5-diphenyl tetrazolium bromide] solution was added to each well after the removal of the culture medium. Incubate the mixture at 37°C for 4 hours. After removing the supernatant, 50 µL of DMSO/ isopropanol was added to each of the wells and incubated for 10 minutes to solubilize the formazan crystals. The absorbance was measured at 620 nm in an ELISA multi-well plate reader (Thermo Multiskan EX, USA). The recorded absorbance was used to calculate the percentage of viability using the following formula:

$$\% \text{ of viability} = \frac{\text{OD value of the experimental sample}}{\text{OD value of the experimental control}} \times 100$$

2.7 Apoptotic analysis by Fluorescent Microscopy

Fluorescence assay was carried out by the methods of Engin et al. (2011). For this, 0.9 ml of cell suspension (1×10^5 cells/mL) was mixed with 1µL of a dye mixture having 100 mg/mL acridine orange (AO) and 100 mg/mL ethidium bromide (EtBr) in distilled water on a clean coverslip. The phosphate-buffered saline (PBS) (pH 7.2) was used to wash pre-treated cancer that was collected. Then, using 10µL of AO/EtBr, the cells were stained; they were incubated for 2 minutes. After incubation, the cells were washed twice with PBS (5 min each) and visualized under a fluorescence microscope (Nikon Eclipse, Inc, Japan) at 400× magnification with an excitation filter of 580nm. The cells were treated with complex for 2 hrs by placing it on a glass coverslip in a 6-well plate. Using 2% Triton X-100 (50µl), the fixed cells were permeabilized for 10 min at room temperature and incubated for 3min with 10µl of DAPI by placing a coverslip over the cells to enable uniform spreading of the stain. The cells were observed under (Nikon Eclipse, Inc, Japan) fluorescent microscope. The MCF-7 cells treated without plant extract were used as a control (Engin et al., 2011).

2.8 DNA Fragmentation assay

DNA fragmentation assay was carried out by the method of Ramar et al. (2012). In 10mM of Tris-Hcl and 10mM EDTA (pH 8.0), MCF-7 (1×10^6cells) were suspended independently. Then, the cells were treated with 10 mM Tris-HCl, 10mM EDTA (pH 8.0), 2% SDS, and 20 mg/mL proteinase K, and the mixture was kept at 37ºC for 3h. Later, 9the mixture was extracted with phenol: chloroform: isoamyl alcohol solution in the ratio of 25:24:1, respectively. The extracted DNA was treated with DNase-free RNase at 20 mg/mL concentration at 4ºC for 45 min and precipitated with 100 mL of 2.5 M sodium acetate and 3 volumes of ethanol. About 10µg of isolated DNA was taken from control and treated cells and electrophoresed on a 2% agarose gel containing ethidium bromide.

3 Results

Results presented in table 2 revealed the phytoconstituents of chloroform, methanol, and ethyl acetate extract of *C. papaya* leaves. The chloroform extract of the *C. papaya* leaves was found to contain alkaloids, saponins, tannins, glycosides, proteins, and carbohydrates. Similarly, methanol extract of the *C. papaya* leaves contains flavonoids, terpenoids, steroids, tannins, glycosides, proteins, and carbohydrates. The ethyl acetate extract of *C. papaya* leaves contains alkaloids, saponins, tannins, glycosides, phenols, anthraquinones, proteins, and carbohydrates.

Figures 2, 3, 4 represent the anti-oxidant activity of the chloroform, methanol, and ethyl acetate extract of the *C. papaya* leaves respectively. Of these extracts, chloroform extract exerted more DPPH free radical scavenging activity than methanol and ethyl acetate extracts, but lesser with the standard ascorbic acid.

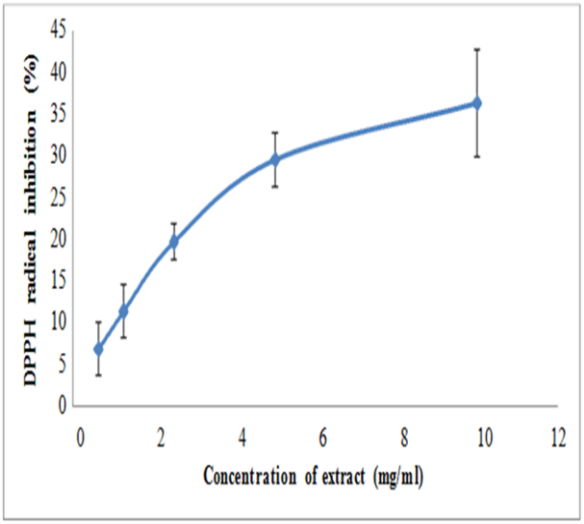

Figure 2 DPPH scavenging activity of chloroform extract of *C. papaya* leaves

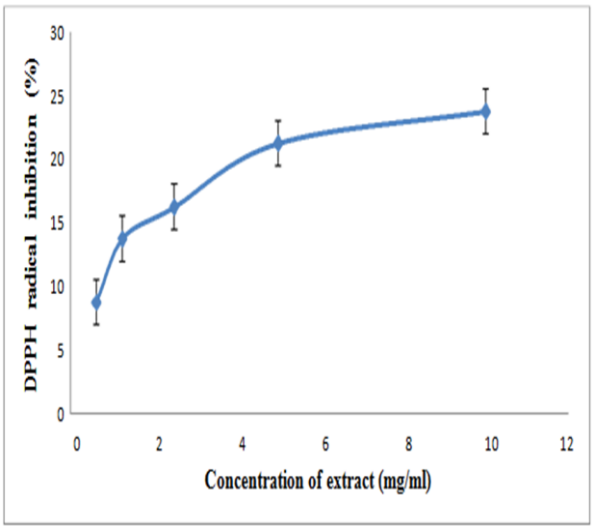

Figure 3 DPPH radical scavenging activity of methanolic extract of *C. papaya* leaves

Table 2 Phytochemical screening of the various extracts of the *C. Papaya* leaves

Phytoconstituents	Chloroform extract	Methanol extract	Ethyl acetate extract
Alkaloids	+	-	+
Flavonoids	-	+	-
Terpenoids	-	+	-
Steroids	-	+	-
Saponins	+	-	+
Tannins	+	+	+
Glycosides	+	+	+
Phenols	-	-	+
Anthraquinones	-	-	+
Protein	+	-	+
Carbohydrates	-	+	+

(+) Presence (-) Negative

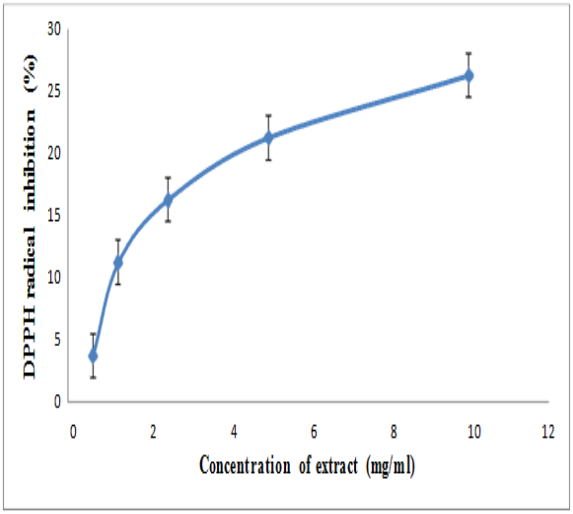

Figure 4 DPPH radical scavenging activity of ethyl acetate extract of *C. papaya* leaves

Figures 5, 6, 7 depict the cytotoxic effect of chloroform, methanol, and ethyl acetate of *C. papaya* leaves at 10µg/ml, 20µg/ml, 40µg/ml against MCF-7 cells. The percentage of viability was analyzed by MTT assay after treatment of *C. papaya* leaves extracts. The IC_{50} values of chloroform, methanol, ethyl acetate extracts on MCF-7 cells was found to be 22±1.5 µg/ml, 30±0.5, and 28±0.5 µg/ml, respectively. Of these three extracts, chloroform extract showed a good cytotoxic effect on MCF-7 cells (22±1.5 µg/ml).

Figure 8 represents the apoptotic effect of chloroform extract of *C. papaya* leaves at 10µg/ml, 20µg/ml, and 40µg/ml on MCF-7 cells and analyzed through fluorescence microscopy. With the increasing concentration of plant extract, a gradual shift from green to red fluorescence was observed in MCF 7 cells, the shift is considered as an index of apoptosis. This is further, supports the apoptotic effect of chloroform extract of *C. papaya* on MCF-7 cells.

Figure 9 represents the DNA fragmentation assay of chloroform extract of *C. papaya* leaves on MCF-7 cells. In the image, "M" represents the marker DNA. The concentrations of the sections were 10µg, 20µg, 30µg, and 100µg, respectively. The DNA fragmentation has increased with the increasing concentration of the extract. Thus, results showed that 100µg of chloroform extract of *C. papaya* leaves exhibit more DNA fragments.

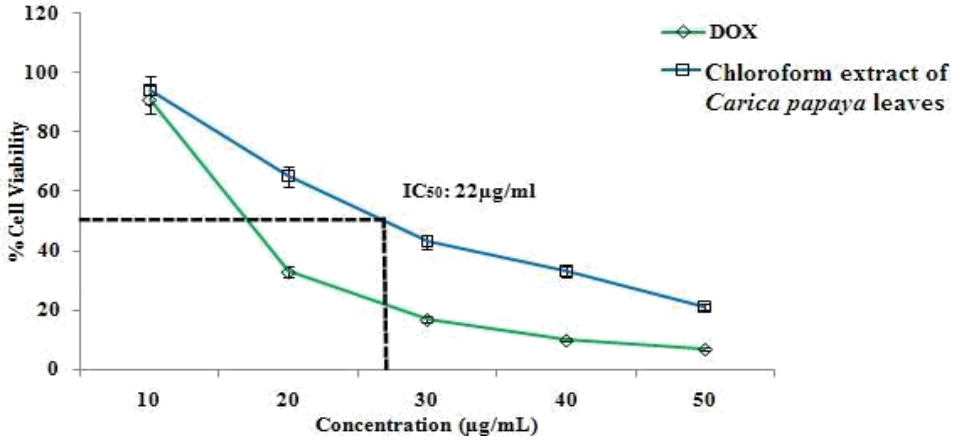

Figure 5 Cytotoxic effect of chloroform extract of *C. papaya* leaves on MCF-7 (MTT Assay)

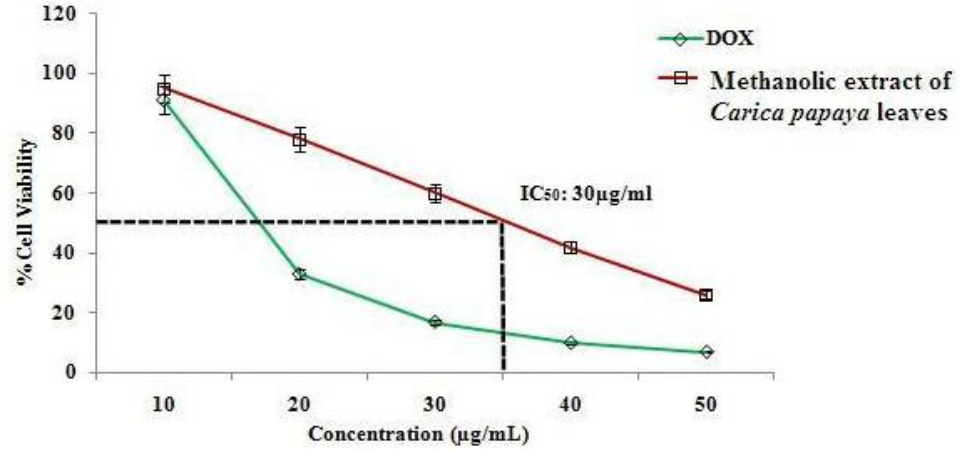

Figure 6 Cytotoxic effect of methanolic extract of *C. papaya* leaves on MCF-7 (MTT Assay)

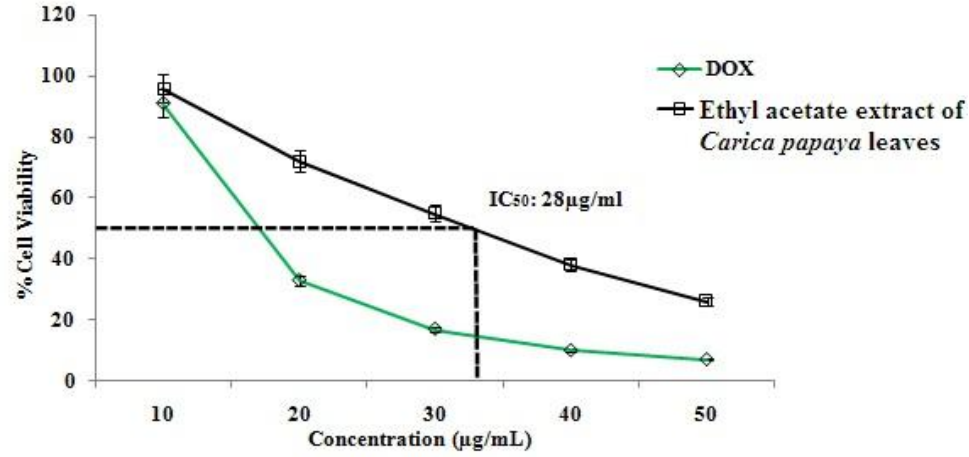

Figure 7 Cytotoxic effect of ethyl acetate extract of *C. papaya* leaves on MCF 7 cells (MTT Assay)

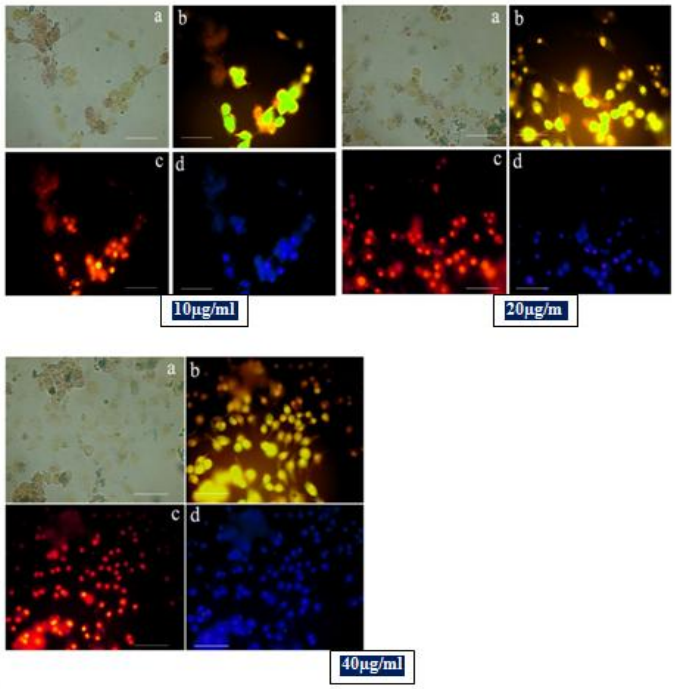

Figure 8 Fluorescence microscopic analysis of chloroform extract of *C papaya* leaves on MCF-7 (a - Phase contrast image of MCF-7-cells; b - Acridine orange and Ethidium bromide image of MCF-7 cells; c - Propidium Iodide of MCF-7 cells; d- DAPI image of MCF-7 cells)

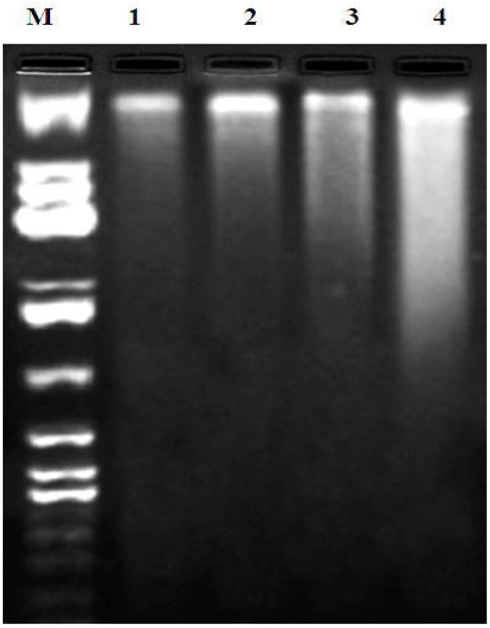

Figure 9 DNA fragmentation analysis of chloroform extract of *C. papaya* leaves on MCF-7 cells (M - Marker DNA; 1 - 10µg/ml treated MCF-7 cells; 2 - 20µg/ml treated MCF-7 cells; 3 -30µg/ml treated MCF-7 cells 4) 100µg/ml treated MCF-7 cells)

4 Discussion

Plants have been used for medical purposes since time immemorial and are the basis of modern medicine. Phytochemicals, secondary metabolites, derived from aerial portions of the plants such as leaves, roots, flowers, seeds, barks, and pulps used to combat several disorders such as asthma, arthritis, cancer, etc. (SahiraBanu & Cathrine, 2015). In this study, leaves of *C. papaya* were found to

possess several secondary metabolites, and it is in line with Javed et al. (2017). It has also been reported that they activate proteins and signaling pathways against cancer cell progression.

Secondary metabolites have been gaining attention as an alternative therapeutic agent against cancer as they act particularly on cancer cells without disturbing normal cells (Rastogi et al., 2016; Javed et al., 2017). Evidence indicates that the free radicals stimulate oxidative damage to biomolecules which ultimately causes aging, diabetes mellitus, inflammation, atherosclerosis, AIDS, cancer, and numerous degenerative diseases in humans. Flavonoids and free radical scavengers that prevent oxidative cell damage possess good anticancer action (Efferth, 2017). In this study, a similar free radical scavenging effect was observed with *C. papaya* leaves and this could be due to the presence of the flavonoids in the plant.

Administration of antioxidants for patients with cancer was found to enhance the therapeutic efficacy and longevity of the patients (Singh et al., 2018). Free radical scavenging ability of chloroform extract was found to be more than methanol and ethyl acetate extracts of *C. papaya* leaves.

Further, the anti-proliferative effect of chloroform extract against MCF 7 was also found to be promising as compared with the other two extracts. Thus, the plant extract possesses antioxidant and cytotoxic effects, researchers suggest that increased free radical scavenging activity, in turn, enhances the cytotoxic effect of plant extract (Suman et al., 2012; Sammar et al., 2019).

Studies on the effect of plant extracts represented major morphological changes, apoptotic characteristics such as shrinkage of the cell, membrane blebbing, and cell density reduced which imply the anticancer activity of the plant extracts (Elumalai et al., 2012; Thangama et al., 2012). The data obtained from the study is in line with previous studies. Similarly, Lu et al. (2014) reported the apoptotic effect of curcumin on MCF-7 cells and observed the characteristic changes of apoptosis. These staining results suggest that the extract triggered apoptosis-mediated cell death in MCF-7.

The DNA fragments generated as the result of apoptosis were visualized after electrophoretic separation. From the results, a 'ladder' pattern of DNA fragments was observed and this is in line with Harshitha et al. (2019). An increased DNA fragment from the MCF 7 cells treated with chloroform extracts further supports the ant-cancer potential of the leaves extract. Previous studies reported that anti-cancer potential may be attributed to either apoptosis induction or DNA fragmentation or caspase -3 activation of poly (ADP) ribose polymerase cleavage caused by these extracts (Desai et al., 2008)

Conclusion

In this study, *C. papaya* leaves extract was selected and screened for its anti-proliferative effect against MCF 7 cells. Qualitative analysis of different extracts revealed the presence of several secondary metabolites which were proven anti-cancer phytoconstituents. From the data obtained, it may be concluded that the *C. papaya* could be used as an alternate anticancer agent and as a good antioxidant.

Conflict of Interest

Nil

References

Alam S, Katiyar D, Goel R, Vats A, Mittal A (2013) Role of herbals in cancer management. The Journal of Phytopharmacology 2(6):46-51.

Chang CC, Yang MH, Wen HM, Chern JC (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 10: 178-182.

Desai AG, Qazi GN, Ganju RK (2008) Medicinal plants and cancer chemoprevention. Current Drug Metabolism 9:581-91.

Efferth T (2017) From ancient herb to versatile, modern drug: Artemisia annual andartemisinin for cancer therapy. Seminars in Cancer Biology 46: 65-83.

Elumalai P, Gunadharini DN, Senthilkumar K, Banudevi S, Arunkumar R, Benson CS, Sharmila G, Arunakaran J (2012) Induction of apoptosis in human breast cancer cells by nimbolide through extrinsic and intrinsic pathway. Toxicology Letters 215(2) 131-142

Engin U, Ceyda A, Ferda A, Eliflkitimur, Yilmaz Y (2011) A Glance at the methods for detection of apoptosis qualitatively and quantitatively. Turkish Journal of Biochemistry 36(3): 261-269.

Fadi MA, Ferguson T (2020) Breast Cancer. A service of the National Library of Medicine, National Institutes of Health.

Farr KJ, Gallaway PJ, Hongu N (2017) Breast Cancer Prevention. Exercise and Healthy Diet. Available at https://extension.arizona.edu/sites/extension.arizona.edu/files/pubs/az1724-2017.pdf access on 29th April 2021.

Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, Ren G (2018) Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes and Diseases 5(2): 77–106.

Harbone JB (1998) Phytochemical Methods. 3rd ed. Chapman and Hill, London.

Harshitha B, Subhada B, Mustafa M, Solanki H, Safiya NAM, Tiwari RVC (2019) DNA laddering to evaluate cytogenetic damage in patients with periodontitis. Journal of International Society of Preventive and Community Dentistry 9: 486-491.

Indumathi CG, Nithyavani DS, Gayathri PK (2014). Estimation of terpenoid content and its antimicrobial property in Enicostemmalitorrale. International Journal of ChemTech Research 6 (9): 4264 – 4267.

Javed I, Banzeer AA, Tariq M, Sobia K, Barkat A, Sayed AS, Ali TK(2017). Plant-derived anticancer agents: A green anticancer approach. Asian Pacific Journal of Tropical Biomedicine 7: 1129–1150.

Joseph A, Sridharan S, Palanisamy S, Ramalingam S, Saravanan R (2020) Identification of anticancer compounds from Linumu sitatissimum seed extract and their effect on HeLa cells. Pharmacognosy Magazine16:221-6.

Kiruthika D, Renuka S, Sheik Abdulla, Shahul Hameed, Sivakumar Ramalingam (2020) Evaluation of Anticancer Potential of Vitus vinifera Seed Against Breast Cancer Cells - MDA-MB-231. Pharmacognosy Journal 12(5): 1064-1071

Kokate CK, Purohit AP, Gokhale SB (2001) Carbohydrate and derived Products, drugs containing glycosides, drugs containing tannins, lipids and protein alkaloids. Text book of Pharmacognosy 7: 133-523

Levitsky DO, Dembitsky VM (2015) Anti-breast Cancer Agents Derived from Plants. Natural Products and Bioprospecting 5: 1-16.

Lu M, Lawrence DA, Marsters S, Acosta-Alvear D, Kimmig P, Mendez AS, Paton AM, Paton JC, Walter P, Ashkenazi A (2014) Opposing unfolded-protein-response signals converge on death-receptor activation to control apoptosis. Science 345: 98.

McDonald S, Prenzler PD, Antolovich M, Robards K (2001) Phenolic content and antioxidant activity of olive extracts. Food Chemistry 73: 73-84.

Mensor LL, Menezes FS, Leitao GG, Reis AS, Santos TCD, Coube CS (2001) Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Research 15 (2): 127-130.

Mustikasari K, Ariyani D (2010) Phytochemical screening of Kalangkala (*Litsea angulata*) seed methanol extract. Sains dan Terap Kim 4(2):131–6.

Padmapriya P, Maneemegalai S (2014) Qualitative and quantitative analysis of the phytochemical constituents of *Mollugo cerviana* (L). International journal of pharmaceutics and drug analysis 2(9): 695-699.

Thangama R, Gunasekaran P, Kaveri K, Sridevic G, Sundarraja S, Paulpandia M, Kannan S (2012) Novel disintegrin protein from Najanaja venom induces cytotoxicity and apoptosis in human cancer cell lines in vitro. Process Biochemistry 47:1243-1249.

Rastogi S, Srivastava N, Virmani T, Singh C, Gupta J (2016) A review on naturally derived compounds for potential anticancer activity. Indian Journal of Drugs 4(3): 75-86.

Richard TS, Kamdje AHN, Mukhtar F (2015) Medicinal Plants in Breast Cancer Therapy. Journal of Diseases and Medicinal Plants 1(1):19-23.

SahiraBanu K, Cathrine L (2015) General Techniques Involved in Phytochemical Analysis. International Journal of Advanced Research in Chemical Science 2(4):25-32.

Sammar M, Abu-Farich B, Rayan I, Falah M, Rayan A (2019) Correlation between cytotoxicity in cancer cells and free radical-scavenging activity: In vitro evaluation of 57 medicinal and edible plant extracts. Oncology Letters 18(6):6563-6571. doi: 10.3892/ol.2019.11054.

Saravanan R, Pemaiah B, Narayanan M, Ramalingam S (2017) Invitro cytotoxic and gas chromatography mass spectrometry studies on *Orthosiphon stamineus* Benth. Leaf asainst MCF-7 cell lines. Asian Journal of Pharmaceutical and Clinical Research 10(3): 1-7.

Saurabh P, Peter JCP, Nicholas S, Amitha KH (2016) Anti-inflammatory and immunomodulatory properties of *Carica papaya*. Journal of Immunotoxicology 13 (4): 590-602.

Schanderl SH (1970) In: Methods in Food Analysis. Academic Press, New York, London, Pp.709.

Shareef M, Ashraf MA, Sarfraz M (2016) Natural cures for breast cancer treatment. Saudi Pharmaceutical Journal 24(3): 233-240.

Silas I, Bolarin JA, Nigeria FI (2019) Qualitative Analysis of Secondary Metabolites in *Cetaurea senegalensis* Plant. International Journal of Innovative Research and development 8 (6): 176-180.

Singh K, Bhori M, Kasu YA, Bhat G, Marar T (2018) Antioxidants as precision weapons in war against cancer chemotherapy induced toxicity – Exploring the armory of obscurity. Saudi Pharmaceutical Journal 26(2):177-190.

Suman S, Pandey A, Chandna S (2012) An improved non-enzymatic "DNA ladder assay" for more sensitive and early detection of apoptosis. Cytotechnology 64(1): 9–14.

Yahyea BL, Romen ML, Pranab BM (2019) Herbal Remedies for Breast Cancer Prevention and Treatment. IntechOpen 10.5772/intechopen.89669.