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Improved micro propagation of Vigna radiata and anticancer activity of in vitro raised plant extract against human breast cancer cell line (MCF-7)

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Abstract

Plants are widely used by all sections of the society either as folk medicines or as pharmaceutical preparation of modern medicine. *In vitro* propagation of plants holds great promise for conservation and enhancement of valuable medicinal plants. *Vigna radiata* has been used in Indian ayurvedic medicine for the treatment of a wide number of health disorders. The present study deals with the influence of different plant growth regulators including shooting and rooting phytohormones on the growth of plant and the nodal segments used as explant were cultured on Murashige and Skoog's medium supplied with different concentration of plant growth regulators. Multiple shoot generation was achieved after 7-8 days of incubation. Methanol extracts showed significant ant proliferative activities in a concentration and time-dependent manner. The inhibitory concentration of methanol extract and acetone extract was tested against MCF-7 cell line, and the results shows that *in vitro* leaf of *Vigna radiata* methanol extract has higher inhibitory effect against the MCF-7 cells at 91.074 g/l than the acetone extract.

Keywords

Microprogation, phytohormones, MS medium, 2,4-D, BAB, MTT assay, MCF-7 cell line, comet assay and Caspase-3

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Contents

1 Introduction **4501**

2 Materials and Methods **4502**

2.1 Materials 4502

2.2 Extract preparation for antibacterial and phytochemical screening 4502

2.3 Cytotoxicity assay of methanol and acetone extract 4502

2.4 Biocompatibility Assay 4502

2.5 Nuclear Staining and Apoptotic Morphology (DAPI and PI Staining Method) 4502

2.6 Determination of DNA Damage by Comet Assay (Single Cell Gel Electrophoresis) 4503

2.7 Protein expression 4503

2.8 Phytochemical Profiling 4503

2.9 Statistical Analysis 4503

3 Results and Discussion **4503**

4 Conclusion **4507**

5 Acknowledgements **4507**

References **4507**

1. Introduction

Medicinal plants are the important bioresource in traditional systems of medicine and are valuable sources of industrially important natural products which includes flavors, fragrances, essential oils, pigments, sweeteners, feed stocks, antimicrobials and pharmaceuticals [1]. Utilizing natural products from medicinal herbs as phytoremedy in the treatment of many diseases and several infection in an age old practices [2]. The long-term availability of many herbs have become uncertain due to indiscriminate exploitation by human and other natural means resulting in possible threat of extinction [3] and the efforts made to cultivate these herbs have met with less fortune. Tissue culture offers the means for rapid and mass multiplication of existing stock of germplasm and also a method for conservation of important, elite endangered plants [4].Tissue culture is the in vitro aseptic culture of cells, tissues,

organs or whole plant under controlled nutritional and environmental conditions, often to produce the clones of plants [5]. The development of tissue culture technology holds great promise for conservation and enhancement of valuable medicinal plants [6]. The decision to propagate medicinal plants using tissue culture depends on the plant part collected, level of threat in the wild, market demand and the quality of transplants for propagation [7]. Micropropagation is an alternative technique for large-scale production of disease free plantlets. It has superiority over conventional method of propagation because of high multiplication rate [2].

Vigna radiata contains plenteous biological activities to prevent human diseases [8]. Mung bean becomes more enriched with metabolites and activities after germination. The beans are rich source of protein and amino acid especially lysine and thus is used as functional food supplement along with cereal-based human diets. It is also a good source of thiamin, niacin, vitamin B6, pantothenic acid, magnesium, iron, phosphorus and potassium, and a very good source of dietary fiber, vitamin C, riboflavin, folate, vitamin K, copper and manganese. It is low in saturated fat and sodium, and very low in cholesterol. Soluble fiber can help lower blood cholesterol [9]. High levels of amino acids, proteins, polyphenols and oligosaccharides in *Vigna radiata* are thought to be the main contributors of the antioxidative, anti-inflammatory, antimicrobial and antitumor activities of this food and play role in the regulation of lipid metabolism [9].

Due to medicinal properties of *Vigna radiata* listed above, the attempts at increasing its population with available alternative methods have recently been increased. As *Vigna radiata* populations have significantly dwindled over the last few decades as a result of degradation of its natural habitats, the possibilities of collecting plant extracts are limited [10]. Research showed that plants grown in sterile in vitro cultures had six times higher levels of active substances than those grown in the wild [11]. However, effective propagation of *Vigna radiata in vitro* requires precise determination of culture conditions, and particlarly mineral composition of the medium. Therefore, the aim of the study was to figure out optimal composition of the medium for in vitro culture of *Vigna radiata* and also to screen the anticancer activities of different solvent extracts of *Vigna radiata* leaves against human breast cancer cell line (MCF-7).

2. Materials and Methods

2.1 Materials

Explants of Vigna radiata seeds, Murashige and Skoog's medium (MS medium), 5% teepol solution, 0.1% mercury chloride, 70% ethanol, 2,4-Dichlorophenoxy acetic acid (2,4-D), Cytokinins, auxin, BAP, MS medium, sterile forceps, scalpels, sterile petriplates, sterile distilled water and culture tubes.

The experiment was conducted in 2008. in the Department of Biotechnology, Jamal Mohamed College, Tiruchirappalli, Tamil Nadu, India. In the first step, plant material was multiplicated to be used further research. The nodal explants were collected from an actively growing mature plant in the field. An initial thorough explants was under tap water to remove the soil particles that remained. The nodules were then washed with dilute detergent solution (5% teepol solution). The explants were then surface sterilized by immersing in 70% ethanol for one minute. After 2-3 distilled water washes, the explants were treated with 0.1% mercuric chloride for one minute in sterile environmental (laminar air flow). The sterilized explants were then inoculated on MS medium supplemented with 0.5-2 mg/l BAB and 0.3-2 mg/l 2,4-D for shoot induction. The plants grew in a phytron under 16 h photoperiod (16 h light/8 h darkness) at 24C. Phytotron shelves were illuminated with 36W cool-light fluorescent lamps generating light with intensity 35 mmol m-2 s-1. Morphological changes were noted and recorded on the basis of observation.

2.2 Extract preparation for antibacterial and phytochemical screening

Fresh leaves of *Vigna radiata* in vitro cultured, field grown micropropagated plants were collected and air dried in closed and dark environment at 40C for 7 days. Dry leaves were powdered in mixture grinder and kept at 4C for further use. The powdered plant material was extracted in two different solvents (methanol and acetone) for 12 h with the help of Soxlet apparatus. Then the extracts were air dried for 24 h and then stored in at 4C for further use.

2.3 Cytotoxicity assay of methanol and acetone extract

Cytoxicity assay MCF-7 cell line was assessed by MTT following the method of Mosmann (1983) [12].

2.4 Biocompatibility Assay

Biocomptability effect of IC50 concentration of methanol and acetone extract of Vigna radiata on vero cells was determined by MTT assay (Mosmann, 1983) to check whether it is toxic to normal cell or not.

2.5 Nuclear Staining and Apoptotic Morphology (DAPI and PI Staining Method)

Nuclear staining was done for MCF-7 cell treated with IC50 concentration of methanol and acetone extract by using DAPI stain and viewing the apoptotic morphology of the cells under a fluorescence microscope, using filters appropriate for DAPI stain by the method of Spector et al. (2001) [13].

2.6 Determination of DNA Damage by Comet Assay (Single Cell Gel Electrophoresis)

Comet assay was performed to document the DNA stability as well as DNA damage in the MCF-7 cells treated with IC50 concentration of methanol and acetone extract following the methodology of Singh et al. (1988) [14].

2.7 Protein expression

Protein expression of Caspase-3 was analyzed according to the method of Towbin et al. (1979) [15].

2.8 Phytochemical Profiling

Best anticancer activity was observed in methanol extract when compared to acetone extract of Vigna radiata and hence the phytocondtituents of methanol leaf extract with its structure was assessed by preliminary phytochemical analysis (Kokate,1988) [16] and FTIR (Shimadzu, IR Affinity 1, Japan).

2.9 Statistical Analysis

The data with five replicates were subjected to statistical analysis and the mean value along with its respective standard error was calculated. The per cent change between control and experimental data were calculated. The data were analyzed statistically using Two Way Analysis of Variance (ANOVA).

3. Results and Discussion

After 7-8 days of incubation small shoots buds were developed from the nodal part of the explants initially later multiple shoots as shown in the fig. 1A-D. These shoots were once again subcultured and transferred to the rooting medium (IBA/IAA). The explants from the plantlet can be made uses for further study.

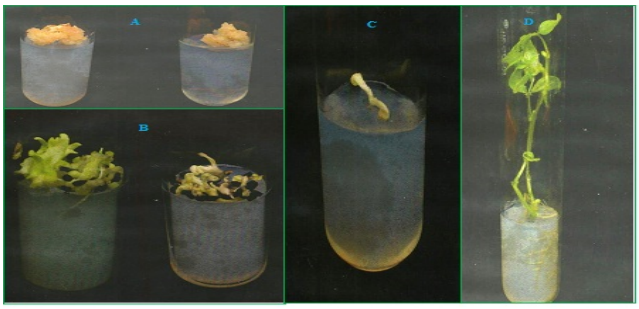

Fig. 1: A- Initiation of callus, B- Regeneration of aseptic plants (Subculture or organogenesis in callus after 7-8 days of culture, C- 5 days of culture after inoculation, D- Indirect organogenesis (Roots were observed after two weeks in the culture tube with 0.5 mg/l BAB. Regenerated plantlets were taken for hardening.

Remarkable variation in respect of regeneration was observed between the varieties in different concentrations of hormone. Among the induced calli, only cotyledon derived calli produced shoots. Hypocotyl and root tip derived calli failed in shoot induction. Shoot tip explants were also cultured for direct shoot regeneration. Anju and Pawan (1992) [17] reported that completew plants were regenerated directly without in intervening callus phase from shoot tips of Vigna radiata on basal medium (MS salts + B5 vitamin). Considering the combined effects of all factors, the highest percentage of shoot (90.00%) was produced in BAB media containing 5.0 mgl-1 BAP and 0.05 mgL-1 NAA from shoot tip explants which required minimum number of days (7-8) for shoot initiation (Fig. 1B).

It was revealed that supplementation of the medium with BAP or Kn alone or a combination of BAP and NAA induced shoots. However, Kn had higher efficiency than BAP while a combination of BAP and NAA was more efficient than the single application of either BAP or Kn. A suitable combination of an auxin and a cytokinin is always considered to be ideal for in vitro morphogenesis from cultured explants. Single application of auxin or cytokinin was reported to regenerate no shoots from garlic root tips [18]. Similar shooting frequency was also reported by Ignacimuthu and Franklin (1998) [19].

Cell viability assay against MCF-7 cell line

The *Vigna radiata* methanol extract and acetone extract were evaluated for their effect on cell viability at concentrations of 25, 50, 75, 100 and 125 g/ml by MTT method against MCF-7 cell line. The tested methanol extract and acetone extract showed a dose dependent decrease in cell viability at the end of 24 hrs. Increasing the time of incubation to 24 hrs showed a further decrease in cell viability (Table 1). Statistical treatment of the data by two-way ANOVA showed that all values were significant at 5% level. Inhibition of cell viability to 50% was observed at 91.074 g/l with an exposure time of 24 h in methanol extract and On the other hand, acetone extract showed 50% inhibition of cell viability at 104.523 g/l in 24 h (Fig.2), and the exact IC50 values are showed in Table 1.

Table 1: Per cent cell viability of MCF-7 cells when treated for 24 h with different extracts of Vigna radiate

Concentration	Methanol extract	Acetone extract
Control (0)	100±00	100±00
25 µg/µl	82.92± 0.899* (-17.08)	91.966±0.652* (-8.034)
50 µg/µl	69.562± 1.035* (-30.438)	85.548±1.073* (-14.452)
75 µg/µl	58.926± 1.731* (-41.074)	63.648±1.214* (-36.352)
100 µg/µl	45.044± 0.612* (-54.956)	52.026±0.534* (-47.974)
125 µg/µl	29.68± 1.011* (-70.32)	40.83±0.819* (-59.17)
IC_{50} µg/µl	**91.074**	**104.523**

Values are mean + S.E. of five individual observations. Values in parentheses are per cent change over control. - Denotes per cent decrease over control.*Values are significant at P¡0.01.

Further studies were carried out at 91.074 g/l of methanol extract and 104.523 g/l of acetone extract; being the 24 h IC50 concentrations. ANOVA analysis revealed that all the values were significantly different. Treatment of MCF-7 cells with methanol extract and acetone extract even at low doses induced morphological changes in the MCF-7 cells, which had similar effect on cells morphology. Microscopic observations were made using Nikon light inverted microscope, wherein treated cells showed distinct cellular morphologi-

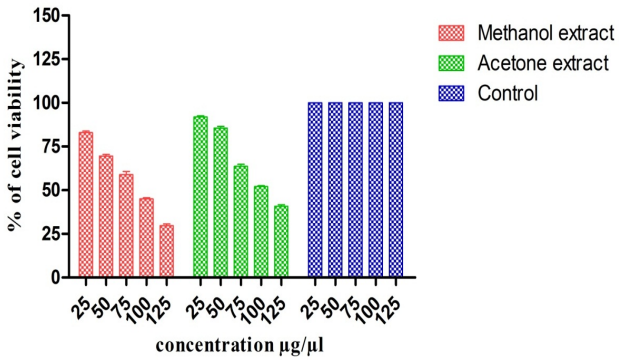

Fig.2. Per cent cell viability of MCF-7 cells when treated for 24 h with different extracts of *Vigna radiata*

cal changes indicating unhealthy cells, whereas the control appeared normal in shape (Fig. 3). Control cells were irregular confluent aggregates with rounded and polygonal cells. This trend is supported by previous studies demonstrating a link between samples with anticancer properties [20-23], as well as evidence to suggest that compounds from *Vigna radiata* may possess anticancer properties [24-27]. The possible mechanism of action of Vigna radiata methanol extract in breast cancer cells was explored by focusing on its ability to induce apoptosis as well as activation of the key apoptosis enzymes, caspases. Our results showed that *Vigna radiata* methanol extract exerted a cytotoxic effect on MCF-7 cell line and inhibited the proliferation of the cells in a dose and time-dependent manner. Inverted microscopic observation also showed that floating cells increased in the breast cancer cells culture treated with *Vigna radiata* methanol and acetone extract, suggesting that *Vigna radiata* has inhibited the growth of MCF-7 cells. Thus, it seems that *Vigna radiata* induces apoptosis in MCF-7 cells through both intrinsic and extrinsic caspase-dependent pathways and, therefore, exerts its anticancer effect.

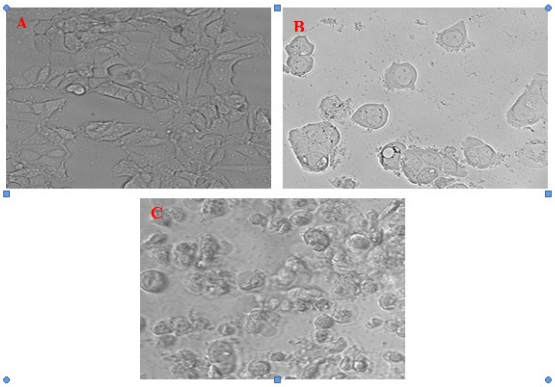

Fig. 3: Cytomorphological image of MCF-7 cells A- Control MCF-7 cells, B- IC50 concentration of methanol extract treated cells and C- IC50 concentration of acetone extract treated cells (10 X magnification).

Biocompatability Assay

Biomedical application of Vigna radiata leaves extract necessitates their biocompatability which is the important criteria to be assessed. On the this basis, cardiomyoblast cells (H9C2) were treated with IC50 concentration of methanol extract and acetone extract for 24 hours. But the both extracts did not cause any cellular changes in the H9C2 cells (Fig. 4). These results demonstrate that methanol and acetone extract of *Vigna radiata* are biocompatible and can be used as a natural drug. Dependable with this concept, DAPI and propidium iodide of the extracts against MCF-7cell lines compared to the H9C2 cell line. *Vigna radiata* is known to be rich in several phytochemicals like alkaloids, flavonoids, tannins, and cardiac glycosides. Some of these secondary metabolities have been reported to possess anticancer activities against different cancer cell lines as opined by Hoet et al. (2004) [28], Fotie et al. (2006) [29], Hong and Kim (2008) [30], Lampiasi et al. (2009) [31], Tang et al. (2010) [32], Akhlaghi et al. (2011) [33], Yadegarynia et al. (2012) [34], Lamchouri et al. (2013) [35], Li et al. (2014) [36], Tor et al. (2014) [37], Maiyo et al. (2016) [38], Du et al. (2017) [39], Yuan et al. (2017) [40] showed that the antiproliferative activities of *Phyllanthus species, Peganum harmala, Dillenia suffruticosa, Holarrhena floribunda, Arachniodes exilis, Croton tiglium, Croton crassifolius, Rosa damascena and Cassytha filiformis,* respectively, were due to their phytochemical constituents.

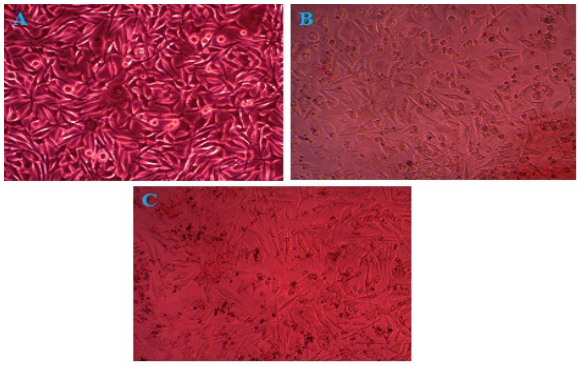

Fig. 4: Cytomorphological image of H9C2 cells A- Control H9C2 cells, B- IC50 concentration of methanol extract treated cells and C- IC50 concentration of acetone extract treated cells (10 X magnification).

Nuclear Staining and Apoptotic Morphology (DAPI Staining Method)

To confirm whether the cytotoxic effect induced by methanol and acetone leaf extract of *Vigna radiata* involves apoptotic changes, the nuclear condensation was studied by the DAPI staining method. In the case of control cells, a variety negligible number of DAPI positive cells were present. In the case of cells treated with 91.074g/l of methanolic leaf extract for 24 hours, a progressive increase in the number of DAPI positive cells was observed when compared acetone extract

treated cells (Figure 5). Apoptosis is characterized by distinct morphological features such as cell shrinkage, chromatin condensation, plasma membrane blebbing, oligonucleosomal DNA fragmentation and finally breakdown of the cell into smaller units (apoptotic bodies). This is the first time we have reported the anticancer activity of *Vigna radiata* against MCF-7 cell line and in future this can be used as a natural anticancer drug to treat many cancer. It is recommended that the *Vigna radiata* due to high nutritional value and antioxidant potential could be used in daily human diet which would be helpful in fulfilling the protein need of the body and helpful in combating cancer.

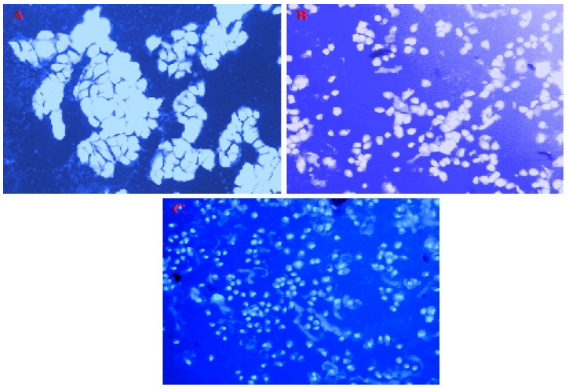

Fig. 5: Nuclear morphology of apoptotic MCF-7 cells A- Control MCF-7 cells, B- IC50 concentration of methanol extract treated cells and C- IC50 concentration of acetone extract treated cells (10 X magnification).

Propidium iodide staining

To confirm whether the cytotoxic effect induced by methanol and acetone extract of *Vigna radiata* involves apoptotic changes, the nuclear condensation was studied by the propidium iodide staining method. In the case of control cells, a very negligible number of propidium iodide postive cells were present. In the case of cells treated with 91.074g/l of methanol extract showed more positive cells than 104.523 g/l of acetone extract for 24 hours as shown in figure 6.

DNA Damage by Comet Assay

As shown in figurte 6 the acetone and methanol extract treated cells showed DNA damage in terms of formation of DNA tail and tail length in cells, while no such changes were observed in untreated control cells. Moreover, it was evidently observed that DNA damage was incubation time-dependent. DNA break was observed in the form of tail formation in extract treated cells when compared with the control. Methanol extract treatment significantly increased the formation of number of tail DNA, tail length and tail movement in MCF-7 cells (Fig. 7 C) than the acetone extract treated cells (Fig. 7 B). The appearance of permeabilized cells labelled with fluorescein conjugate suggests that the methanol extract were internalized and resided in the cytoplasmic region of the MCF-7 cells when

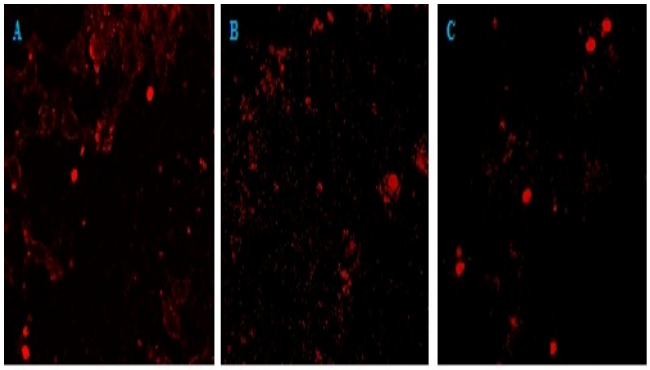

Fig. 6. Morphology of apoptotic cells stained with propidium iodide A- Control MCF-7 cells, B- IC50 concentration of methanol extract and C- IC50 concentration of acetone extract

compared to the control as well as acetone extract treated cells (Fig. 7B). The results illustrates that more DNA damage was observed in methanol extract treated MCF-7 cells when compared to acetone extract treated cells, which is well depicted by head and tail DNA content. In contrary, in the control MCF-7 cells, the DNA remained intact without any damage in it.

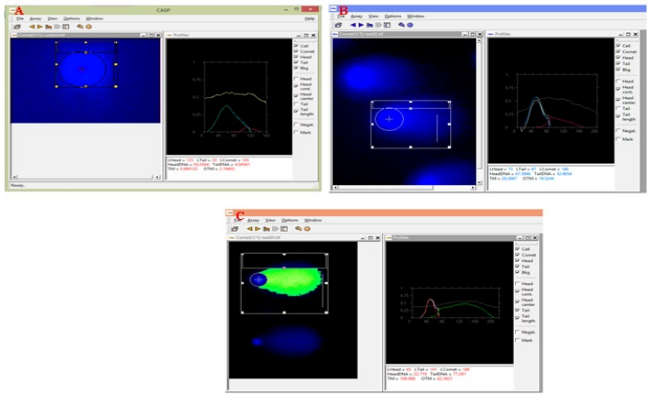

Fig. 7: Analysis of DNA damage of MCF-7 cells when treated with 24 h IC50 concentration A - Control MCF-7 cells, B - IC50 concentration of acetone extract and C- IC50 concentration of methanol extract

Methanol extract might encourage programmed cell death than acetone extract through p53 balancing the ratio of Bax to Bcl-2. Bcl-2 family is one of major classes of regulators in the intrinsic pathway in our study similar observation were also observed by Chang et al. (2006) [41] in DU145 and LNCaP prostate cancer cells.

From the above study methanol extract alone showed good anticancer property and the possible anticancer mechanisms of methanol extract, the expression of apoptotic genes was analysed. After treatment with methanol extract, expression of caspase-3 was increased (Fig. 8). The results obtained

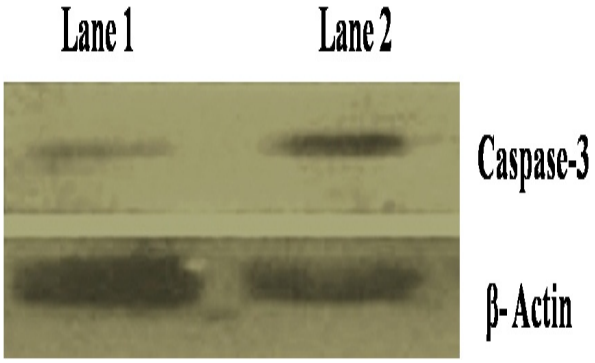

Fig. 8: Effects of methanol extract on Caspase-3 expression in MCF-7 cells. Lane 1- Control MCF-7 cells and Lane-2 - cells incubated with IC50 concentration of methanol extract and then Caspase-3 expression were analyzed using western blots by use of the corresponding specific antibodies.

in this study indicated that methanol extract induced programmed cell death through the mitochondrial apoptotic pathways, which was confirmed by the observed enhanced expression levels of caspase-3 after treatment. These pro- and antiapoptotic genes have been the main regulators of the intrinsic pathway of cell death. Based on the above activity exhibited by the methanol extract, identification of active substances would play a significant role in the safe, effective use of methanol extract therapeutically. In the end, the current study confirmed that the methanol extract killed MCF-7 cells by induction of apoptosis.

Preliminary phytochemical analysis

The phytochemical characteristics of *Vigna radiata* methanol extract revealed the presence of acids, alkaloids, anthocyanins and betacyanins, carbohydrates, cardiac glycosides, glycosides, proteins, coumarins, flavonoids, phenols, quinones, tannins, terpenoids and triterpenoids. On the other hand, saponins and steroids were absent in the methanol extract (Table 2).

Table 2: Preliminary phytochemical analysis of *Vigna radiata* methanol extract

S. No.	Phytocompounds	Presence/Absence
1.	Acids	+++
2.	Alkaloids	++
3.	Anthocyanins and Betacyanins	+
4.	Carbohydrates	++
5.	Cardiac Glycosides	+
6.	Coumarins	++
7.	Flavonoids	+++
8.	Glycosides	+
9.	Phenols	+++
10.	Proteins	+
11.	Quinones	+
12.	Saponins	-
13.	Starch	+
14.	Steroids	-
15.	Tannins	+
16.	Terpenoids	+
17.	Triterpenoids	+++

+++ Strongly present, ++ mildly present, + present , - absent

+++ Strongly present, ++ mildly present, + present, - absent

FTIR Spectral analysis

FT-IR spectrum showed the presence of C-F stretch (Alkyl Halides, strong), monosubstituted alkene and CC stretch (variable) as shown in Fig. 9

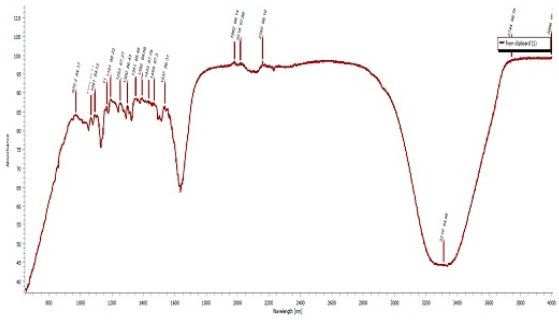

Fig. 9: FTIR spectrum of *Vigna radiata* methanol extract (800 to 4000 cm-1)

FTIR spectroscopy is capable of providing strong insight into the structural and functional alterations induced by various factors due to its high sensitivity [42]. FTIR technique was used for assessment the type of organic and inorganic complexes in methanol extract. The present study, the FTIR spectroscopic analysis showed the presence of phyto constituents in the methanol extract. The result in the present study showed that FTIR spectroscopy is a valuable technique to fingerprint and to analyze the different biomolecules from methanol extract which contains 13 peaks. Based on the peak values more functional groups were obtained from the methanol extract. In order to validate the FTIR method as a good tool to investigate fingerprint and to predict the composition of the methanol extract and to evaluate the quality and its detoxifying capacity of *Vigna radiata*. The result of the FTIR analysis is contradictory to the results Feng et al. (2009) [43] and Muthukrishnan et al. (2015) [44]. The results of the present study confirmed that *Vigna radiata* may be wealthy resource of phytoconstituents which can be isolated and examined for further pharmacological activities. Taking into account of all the results of the present investigation, ethanopharmacological approach plays a promising role in the development of cancer therapy. Results of the present investigation reveals that excessive reactive oxygen species (ROS) generation might have played a dominant role. This ROS generation might be due to the presence of phytomolecules in *Vigna radiata,* thus inducing cancer cell apoptosis. The generated ROS favors the DNA damage leading to apoptosis. Also this is widely accepted concept, but limited in application, due to lack of available technologies and process of validation. Our study investigated the anticancer activity of *Vigna radiata* leaves extracr as a whole (crude). The preliminary screening studies on phytochemical constituents present in the extract possess anticancer and anti-apoptotic activity.

4. Conclusion

In conclusion, for the creation of genetic variability in crop plants it is very important to regenerate plants via callus. With this obvious reasons it is suggested that in future tissue culture programme of mungbean some more cytokinin's additives viz., zeatin, coconut water, ascorbic acid, coelonic fluid of earthworm, vermiwash and glutamine may be used for successful plantlet regeneration from calli. Here a direct in vitro regeneration protocol was developed. However, further study is needed with different explants to standardize the protocol of regeneration from calli. The protocol developed here could be used for future improvement of *Vigna radiata* through in vitro culture. Methanol extract of *Vigna radiata* leaves demonstrates promising anticancer and anti-apoptotic properties against human breast cancer cell line (MCF-7) by in vitro method. Increasing awareness, promotion and utilization of this plant for public benefits are highly encouraged and identification of active phytoconstituents in the leaves will serve as a natural cytotoxic agent against various cancers.

5. Acknowledgements

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References

[1] Badoni. A, Bisht. C. and Chauhan. JS. Micropropagation of Hedychium spicatum Smith using in vitro shoot tip stem cell. Plant Cell Tiss. Org. Cult.,1: (2010).11-13.

[2] Deventhiran. M, John Wyson. W, Sheik Noor Mohamed. M, Jaikumar. K, Saravanan. P. and Anand.D. In vitro propagation and comparative phytochemical analysis of wild plant and microprogated Cleome rutidosperma DC. IJPPR., 9(2): (2017).253-257.

[3] Razdan. MK. Introduction to plant tissue culture. (2nd Ed.), Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India. (2003). pp.27.

[4] Ncube. NS, Atolayan. AJ, Okoh. AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. African J Biotech., 7: (2008).1797-1806.

[5] Thorpe. T. . History of plant tissue culture. J Mol. Microbiol. Biotech., 37: (2007), 169-180.

[6] Jeyachandran. R, Baskaran Xavier. R and Cindrella. L. An efficient in vitro plant regeneration of Dipteracanthus prostratus. Asian Pacific J Trop. Biomed., (2012). 484-487.

[7] Botha. J, Witkowski. ETF and Shackleton. CM. The impact of commercial harvesting on Warburgia salutaris in Mpumalanga, South Africa. Biodiversity and conservation. 13:(2004), 1675-1698.

[8] Sies, H. Oxidative stress: Oxidants and antioxidants. Exp. Physiol. 82: (2001)291-295.

[9] Van Horn, L. and Ernst, N. A summary of the science supporting the new National Cholesterol Education Program dietary recommendations: What dietitians should know. J Am Diet Assoc. 2: (1997). 1148-1154.

[10] Tang. D, Dong. Y, Ren. H, Li, L. and He. C.. Metabolomic analysis of the polyphenols in germinating mung beans (*Vigna radiata*) seeds and sprouts. J Sci Food Agric. 94(8):(2014) 1639-1647.

[11] T Krenn, T. and Kartnig, T. Phytotherapie. Frank furt.1(2005). 97-202.

[12] Wawrosch, C. (An improved two step liquid culture system for efficient in vitro shoot proliferation of Sundew. Sci. Pharm. Vienna., 2005). 251-262.

[13] Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65(2): (1983). 55-63.

[14] Spector, DL., Auman, RD. and Leiward, LA. Cell culture analysis: Apoptosis analysis. In: "Cell - A Laboratory Manual". (4th ed.), (Eds.), Coldspring Harbour Laboratory Press, New York, USA.(2001). pp. 6-15.

[15] Towbin, H., Staehelin, T. and Gordon, J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Nat. Acad. Sci.,76(9): 4(1979). 350-4354.

[16] Singh, NP., McCoy, MT., Tice, RR. and Schneider, EL. A simple technique for quantification of low levels of DNA damage in individual cells. Exp. Cell Res., 175(1):(1988). 184-191.

[17] Kokate, CK. In: "Practical Pharmacognosy". (4th edn.), (Ed.), Vallabh Prakashan, New Delhi, India. (1988).pp. 4-29.

[18] Anju, G. and Pawan. KJ. In vitro induction of multiple shoots and plant regeneration from shoot tips of mungbean. Plant Cell Tiss. Org. Cult., 29(3): (1992). 199-205.

[19] Haque, MS., Wada, T. and Hattori, K. High frequency shoot regeneration and plantlet formation from root tip of garlic. Plant Cell Tiss. Org. Cult., 50: (1997). 83-89.

[20] Ignacimuthu, S. and Franklin, G. Regeneration of plantlets from cotyledon and embryonal axis explants of vigna mungo. Plant Cell Tiss. Org. Cult., 55(1): (1998). 75-78.

[21] Manimekalai, I., Sivakumari, K., Ashok, K. and Rajesh, S. Antioxiodant and anticancer potential of mangosteen fruit, Garcinia mangostana against hepatocellular carcinoma (HepG-2) cell line. World. J. Pharm. Sci., 5(2): (2016b). 253-293.

[22] Rajesh, S., Sivakumari, K., Ashok, K. and Abitha, AR. Anticancer activity of Cardiosprmum halicacabum leaf extracts against hepatocellular carcinoma cell line (HepG-2). World. J. Pharm. Sci., 5(3):(2016). 1133-1154.

[23] Jayaprakash, P., Sivakumari, K., Ashok, K., Rajesh S., Prabhu D. and Chandrasekar, D. Anticancer potential of green synthesized silver nanoparticles of Sargassum wightii againts human prostate cancer (PC-3) cell line. Ejpmr., 4(3): (2017). 275-287.

[24] Saranya, A., Sivakumari, K., Ashok, K. and Rajesh, S. Phytochemical profiling and anticancer study of lyophilized pure fruit juice of Citrus limon (L.) Osbeck against human breast cancer (MCF-7) cell line. J Adv. Mol. Bio., 1(2): (2017).91-103.

[25] E.W. Thompson, M. V. Laycock, J. A. Ramshaw, and D. Boulter, "The amino acid sequence of Phaseolus aureua L. (mung-bean) cytochrome c.," Biochemical Journal, 117(1), (1970). pp. 183–192.

[26] A. Adel, Y. Shehata, and A. M. Thannoun, "Chemical and amino acid composition of Iraqi mung beans," Zeitschrift fur Lebensmittel-Untersuchung und Forschung, vol. 171, pp. 360– 362, 1980.

[27] N. Bhatty, A. H. Gilan, and S. Nagra, "Nutritional value of mung bean (*Vigna radiata*) as effected by cooking and supplementation," Archivos Latinoamericanos de Nutricion , vol. 50, no. 4, (2000). pp. 374–379.

[28] Khatun, M. K., Haque, M. K., Islam, S., Nasiruddin, K. M., In vitro regeneration of mungbean (*Vigna radiata L.*) from different explants. Progress. Agric. 19(2),2008. 13-19.

[29] S. Hoet, C. Stevigny, S. Block et al., "Alkaloids from Cassytha filiformis and related aporphines: antitrypanosomal activity, cytotoxicity, and interaction with DNA and topoisomerases," Planta Medica, 70(5), (2004).pp. 407–413,

[30] J. Fotie, D. S. Bohle, M. L. Leimanis, E. Georges, G. Rukunga, and A. E. Nkengfack, "Lupeol long-chain fatty acid esters with antimalarial activity from Holarrhena floribunda," Journal of Natural Products, 69(1), (2006), 62–67.

[31] Hong, E.; Kim, G.H. Anticancer and antimicrobial activities of -phenylethyl isothiocyanate in Brassica Rapa L. Food Sci. Technol. Res. (2008), 14, 377.

[32] N. Lampiasi, A. Azzolina, N. D'Alessandro et al., "Antitumor effects of dehydroxymethylepoxyquinomicin, a novel nuclear factor-B inhibitor, in human liver cancer cells are mediated through a reactive oxygen species-dependent mechanism," Molecular Pharmacology, vol. 76(2), (2009). pp. 290–300,

[33] Y.-Q. Tang, I. B. Jaganath, and S. D. Sekaran, "Phyllanthus spp. induces selective growth inhibition of PC-3 and mewo human cancer cells through modulation of cell cycle and induction of apoptosis," PLoS ONE, 5(9), e12644, 2010.

[34] Akhlaghi AZ, Rakhshandeh H, Najaran ZT, Mousavi SH Study of cytotoxic properties of Rosa damascena extract in human cervixcarcinoma cell line. Avicenna J Phytomed (2011),1:74-77.

[35] S. Yadegarynia, A. Pham, A. Ng et al., "Profiling flavonoid cytotoxicity in human breast cancer cell lines: determination of structure-function relationships," Natural Product Communications, 7(1), (2012).pp. 1295–1304,

[36] F. Lamchouri, M. Zemzami, A. Jossang, A. Settaf, Z. H. Israili, and B. Lyoussi, "Cytotoxicity of alkaloids isolated from Peganum harmala seeds," Pakistan Journal of Pharmaceutical Sciences, 26(4), (2013). 699–706.

[37] H. Li, J. Chen, C. Xiong, H. Wei, C. Yin, and J. Ruan, "Apoptosis induction by the total flavonoids from Arachniodes exilis in HepG2 cells through reactive oxygen species-mediated mitochondrial dysfunction involving MAPK activation," EvidenceBased Complementary and Alternative Medicine, vol. 2014, Article ID 906941.

[38] Y.S. Tor, L. S. Yazan, J. B. Foo et al., "Induction of apoptosis through oxidative stress-related pathways in MCF-7, human breast cancer cells, by ethyl acetate extract of Dillenia suffruticosa," BMC Complementary and Alternative Medicine, 14, 55, (2014).

[39] Maiyo, F.C.; Moodley, R.; Singh, M. Cytotoxicity, antioxidant and apoptosis studies of quercetin-3-O glucoside and 4-(-D-glucopyranosyl-14--L-rhamnopyranosyloxy)-benzyl isothiocyanate from Moringa oleifera. Anticancer Agents Med. Chem. (2016), 16, 648–656.

[40] Du, Q.; Zhao, Y.; Liu, H.; Tang, C.; Zhang, M.; Ke, C.; Ye, Y. Isolation and structure characterization of cytotoxic phorbol esters from the seeds of Croton tiglium. Planta Med. (2017), 83, 1361–1367.

[41] Yuan, Q.Q.; Tang, S.; Song, W.B.; Wang, W.Q.; Huang, M.; Xuan, L.J. Crassins A-H, diterpenoids from the roots of Croton crassifolius. J. Nat. Prod. (2017), 80, 254–260.

[42] Chang HK1, Shin MS, Yang HY, Lee JW, Kim YS, Lee MH, Kim J, Kim KH, Kim CJ. (2006). Amygdalin induces apoptosis through regulation of Bax and Bcl-2 expressions in human DU145 and LNCaP prostate cancer cells. Biol Pharm Bull. 29(8)(2006), 1597-602.

[43] Severcan, F., Toyran, N., Kaptan, N., Turan, B., Fourier transform infrared study of the effect of diabetes on rat liver and heart tissues in the C–H region. Talanta 53, (2000). 55–59.

[44] Feng N, Guo X and Liang S. Adsorption study of copper (II) by chemically modified orange peel. J. Hazar Mater, (2009), 164:1286–1292.

[45] Muthukrishnan S. Bhakya S. Senthil Kumarc T. and Rao. M.V. Biosynthesis, characterization and antibacterial effect of plant-mediated silver nanoparticles using Ceropegia thwaitesii – An endemic species. Industrial Crops and Products; (2015), 63:119– 124.

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