IN-VITRO METHODS OF SCREENING OF ANTI-CANCER AGENT

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ABSTRACT

Global cancer population is more than 6.75 billion, and today cancer is the leading cause of mortality. Medical therapy in cancer treatment is an area of investigation for which efforts are being made to come up with novel chemical entities for better treatment of cancer. Discovery of new anti-cancer drugs is based on a mechanistic approach and has reliance on empiric cell-based screening of new drugs for its anti-cancer effects. To summarize the data related to current in-vitro methods for screening of anticancer agents. Reviewed here are some selected In-vitro cell based methods for screening of anti-cancer drugs. These are the combinations of systematic methods based on existing theories and models. New anti-cancer drug is necessary with novel profile of anti-tumor activity. This review will summarize most popular in-vitro cell based screening methods, which are economically good enough to establish the anti-cancer activity of newly evolved anti-cancer drugs.

KEYWORDS: In-Vitro Screening Methods, Cell Based Assay, Anti-Cancer Agent, Tetrazolium Salt, Colorimetric Assay, DNA Fragmentation.

1. INTRODUCTION

Cancer is the enormous type of disease which is involve in the abnormal cell growth with the dormant to spread to other parts of body. They form a set of tumor or neoplasm, A neoplasm or tumor is a group of cells, which is also called malignancy, have undergone unregulated
growth of cell and often form a mass and lump, which may be invade diffusely.[1,2] The various number of anticancer agents with sales of $ billion or more has increased from past decade. Again, the need for novel drugs with the novel mechanism of action is still pressing. Public institutions, the pharmaceutical industries, and biotech companies invent lots of compounds with desired anti-cancer activity. Only assertive numbers of drugs and concepts of action evaluated clinically because of cost and other ethical considerations. Therefore a pre-selection, called the screening process is required to identify products that will able to perform anti-tumor effects.[3]

Various types of treatments are available for cancer patients like surgery, chemotherapy, radiations, etc. But there are lots of side effects related to these treatments, hence now a days scientist are focussing towards development of new anticancer drugs. The biological evaluation of these newly synthesize compounds include various in-vitro or in-vivo techniques. Direct screening by in-vivo technique requires lot of expenses and approval from animal ethics committee hence it is always better to screen the synthesized drugs by various in-vitro techniques which are cheaper. After screening by in-vitro technique, the promising compounds can be screened further by in-vivo technique.[3] Considering our present article, we have discussed principle of various in-vitro cell based technique which are available for evaluation anticancer.

Cell based or In-vitro screening assays are often used for the screening and collection of compounds to identify the test compounds which shows direct cytotoxic effects or have effect on cell proliferation , that eventually lead to cell death. Cell-based depend assays are mostly used for amplification of receptors binding and different signal-trasductions events, which may also comprise as an expression of genetic receptors, trafficking of cellular components, or to moniter orgenelle function. Several researches have been conducted to estimate the toxicity of medicinal plant.[2]

1.1 IN-VIVO AND IN-VITRO SCREENING

Large-scale screening using animal systems as practiced in the past decade is highly unethical and strictly regulated. In the majority of cases, either cellular or target-based high-throughput assays will precede in-vivo evaluation of potential anticancer drugs. High-throughput screening (HTS) plays an essential role in contemporary drug discovery processes. The screening and evaluation of the compound by in vitro and in vivo animal models are important tools in cancer research which will enabling the identification of carcinogens, the
development of cancer therapies, drug screening, and providing insight into the molecular mechanisms of tumor growth and metastasis.\cite{3} Presently, active compounds are selected by prescreening and screening against transplanted mouse tumors and human tumor xenografts as well as by the \textit{in-vitro} systems. Because of ethical, medical and economical limitations and constraints on the number of patients eligible for clinical trials, most of the research has been done in experimental system. The traditional anticancer drug screening methods, including animal experiments and cell-based screening assays.\cite{3}

Most pre-clinical data on new anticancer drugs were obtained using transplanted tumors in mice. For practical reasons, scientists mainly use ectopically-implanted, subcutaneously-growing tumor models, frequently as xenografts of human origin. Considerable scepticism about the value of fast growing Animal tumor systems have to meet several requirements to be suitable for experimental tumor therapy. It is very important that the tumor precisely reflects treatment response, and that the natural history of the host allows the study of the experimental endpoint, e.g. a sufficient life-span for follow-up to assess local tumor control. An enormous variety of different tumor systems for in vivo evaluation of new anticancer agents is available.\cite{4} Mostly murine host systems are used for experimental tumor therapy because of the availability of in-bred lines at relatively low costs. \textit{In-vitro} models to define the mechanisms of action of a given compound Once a compound has demonstrated robust cytotoxic activity against a panel of human cancer cell lines and deserves further investigation in \textit{in-vivo} models, it is important to clarify its mechanism of action and to identify its exact molecular targets. Over many decades, researchers in experimental tumor therapy have developed well-proven, reliable in vitro and in vivo methods to evaluate treatment response. The use of these standardized experimental methods is time-consuming and costly, and there is, consequently, a gap between the quantity of new agents and the resources available for their evaluation.\cite{5}

1.2 NEED OF \textit{IN-VITRO} SCREENING

The various approaches for the invention of new anti-cancer drugs has recently emerged from a reliance on cell-based screening for anti-cancer effects, towards more mechanistically based approaches, that targets the specific molecular defect which thought to be responsible for the development and maintenance of the malignant phenotype in various forms of cancer.\cite{4,5} The ultimate goal of the development of molecular targeted drugs is to improve the efficacy and selectivity of cancer treatment. Commonly used cytotoxic anti-cancer drugs were discovered
through random high-throughput screening (HTS) of synthetic and natural products in cell-based cytotoxic assays. Despite the chemical diversity of these agents, the mechanisms of action are limited and most compounds are DNA-damaging agents with a low therapeutic index. With these screening approaches, the mechanism of action is not a primary determinant for selecting a drug for further processes, and as a result, none of the drugs is not get directly targets the molecular lesions which are responsible for malignant transformation.

Initial screening for drug development programs were small in scale and directed towards the evaluation of anti-tumor activity of small number and specific types of potential drugs. Compared to animal tumor models, in-vitro methods are less time-consuming and less expensive, thereby allowing the evaluation of large quantities of new anticancer agents. Sophisticated in-vitro experiments provide data related to mechanisms of action, which when combined with detail characterization of tumor cell lines, to identify tumor entities which may respond to the drug. Selection of anti-cancer drugs for in-vivo testing requires the data which is obtained from functional assays. Thus, quantitative information obtained from in-vitro evaluation of drugs is use for further testing in animal models. Recent developments, such as co-culture models and use of genetically manipulated cell lines has improved for studying the mechanisms which reflect the drug effect on cancer cells.

1. Methods for in-vitro screening

1. MTT Assay: (Colorimetric Assay Based Upon Tetrazolium Salt)

The amount of colour produce by a specific reagent in the assay can be measure by calculating amount of light absorbed by the compound or newly form product at particular wavelength, this is called colorimetric base assay. It is a type of method which determines the concentration of a chemical element or chemical compound in a solution with the aid of a colour reagent. Micro culture tetrazolium salt based assays (MTAs) are colorimetrically based assay, they based on the reduction of a tetrazolium salt by a mitochondrial enzyme, which lead to the production of a coloured compound which is called formazan, quantified by spectrophotometry.

Types of Tetrazolium Salts

1. MTT, a yellow coloured tetrazole, which was converted into purple formazan in the cell. The formazan is insoluble in the medium which could be solublize by the use of solution, which make the medium clear. The quantification is done by taking absorbance of this
solution at specific wavelength, normally it is taken at 500 and 600 nm by the use of spectrophotometer.\cite{6}

2. **XTT** (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) has been processed to repulse into other tetrazolium salt asssy, to yield more sensitive and greater effective range. The advantage of this assay is, obtained formazan product is water-soluble, which will avoid final addition of solubilising solvent.\cite{6}

3. **MTS** (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), by the addition of phenazine methosulfate (PMS) obtained formazan product has an maximum absorbance at 490-500 nm with the use of phosphate-buffered saline. The assay has usually known as a 'single-step' MTT assay, which will offers us for straight addition of cell culture without the other additional steps, which is essential in the MTT assay. However this convenience makes the MTS assay sensitive to colorimetric interference.

4. **Wsts** (Water-soluble Tetrazolium salts) 8(2-(methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium), this assay has been developed to obtained different absorption spectra of the formed purple coloured formazan. The assay has an advantageous over other salt based assay is that they are reduced outside cells, combined with already added PMS solution which behave as a electron mediator, and produce a water-soluble formazan. Water-soluble tetrazolium salts are more recent alternatives to MTT.\cite{6}

**Figure. 1.**

**MTT assay** is a type of colorimetric assay, which had been extensively used for the estimation of the cell metabolic activity. Under certain specific conditions NADPH-dependent cellular oxido-reductase enzymes may, equal to the number of viable cells present in the medium.\cite{7} They are able to reduced tetrazolium dye into its purpple coloured formazan product. Different type of salt based assays including XTT, MTS and the WSTS are used in unification with the addition of intermediate electron acceptor, 1-methoxy phenazine
methosulfate (PMS). MTT assays can also be used to quantitate loss of viable cell(cytotoxicity) and shifting of proliferation to quiescence (cytostatic) by the use of potential medicinal agents and toxic materials. The alteration of MTT by cells in culture is time dependent. The accumulation of colour can be happen if incubation time will increase and sensitivity is also affected. Because of the toxic nature of the reagents, the incubation time should be limited otherwise they will the incubation time is limited because of the toxic nature of the detection reagents which promote to the energy from the cell to generate a signal(reducing equivalents such as NADH).[7]

These salt based assays are usually done in the dark since the salt is sensitive to light. the cell get lose there ability to produce MTT they die, and color formation serves as a essential and convenient marker for the viable cells.

2. SRB assay (sulforhodamide-b assay)
Since from decade the two major types of assay or techniques were used to evaluate the cell mediated growth. The most common or earliest used methods are either MTT or XTT. The basic principle of these reagents is relies on the formation of color compound, that depend on the activity of the mitochondria. So variable results can be obtained if the variations is done by the cellular levels of glucose or other cellular content or functions of these reagents is inhibited and if the cells were not alive or not proliferating then similar result has been shown.[8] These types of limitations can be overcome by the second major technique for testing cell viability or cytotoxicity is the sulphorhodamine b (SRB) assay, which is more preffered.

SRB is an anionic dye and it is aminoxanthene, which can react with basic amino acid residues of protein to forms an electrostatic complex under moderately acid conditions, which contribute to a susceptible and linear response. The cells get prepare by thoroughly washed it, fixed and stained it with the dye. The reagents which were incorporated get liberated from the cells with a tris base solution. The formation of color is quick and stable and the absorbance can be readily measured at wavelength between 560 and 580nm. The concomitant change in the amount of dye which is incorporated in the culture contribute to the increase or decrease in the total number of cell. These changes will show the degree of cytotoxicity or cell viability caused by the test compound. These all evaluation is depend on the uptake or incorporation of the pink aminoxanthine (negatively charged) dye by amino acids (basic) in the cells.[8] The greater amount of dye is taken up by the cell if adequate amount of cell will
present and after washing and fixing, the released dye will give a more acute color and greater absorbance when get lysed. It is rapid, susceptible, sensitive, and inexpensive method for measuring the cellular protein content of the cell.

3. Clonogenic Assay

The clonogenic assay determines cell proliferation, it is an *in-vitro* type of cell survival assay. The ability of a cell to proliferate indefinitely is said to be clonogenic, with the ability to form a reproductive and large colony or a clone. The principle need of the assay is the ability of a cell to raise into a colony. The set of atleast 50 cell is defined as colony. The ability of a single cell to grow into a large colony that can be visualized with the naked eye is proof that it has retained its capacity to reproduce. The assay will determine the effectiveness of cytotoxic agents, it can also evaluate the cell reproductive death after programming with ionizing radiation.\(^9\) Although initially the clonogenic assay have played an essential role in radiobiology, by studying the effects of radiation on cells. Now they are widely use to examine the effect of ionizing radiation on cell or chemotherapeutic agents such as etoposide, etc, or anti-angiogenic agents such as endostatin, and cytokines and their receptors, either alone or in combination therapy.

![Figure 2](image)

The clonogenic cell survival assay has able to detect all cells which retained the capacity for producing a large number of progeny after treatments that can cause cell death as a result of damage to chromosomes which lead to apoptosis. In addition to the other methods for cells from culture and cells from normal tissues, clonogenic assays have been developed for cells from tumors in animals.\(^9\) Cells in transplantable tumors are harvested to get cell suspensions, after that known numbers of cells are injected into recipient animals where they can develop further into new tumors. If donor tumors are become irradiated before harvesting, then the fraction of the cells will lose clonogenic capacity and, as a consequence, larger numbers of
tumor cells are required to yield tumors in recipient animals. Many studies performed with all these systems which give information about the differences in to the sensitivity related to the radiation and chemotherapeutic agents among tumors and normal tissues and also about the modification of treatment effectiveness by various conditions and modes of application.\[^{9}\]

The capacity for continued proliferation of stem cells in tissues is required for the continue integrity and for function of normal tissues, whereas in tumors eradication, the capacity for unlimited proliferation of all stem cells is required for the prevention of recurrences.

4. **Thymidine Incorporation Assay**: This assay is the most common assay which utilizes a strategy by the used of radioactive nucleoside, 3H-thymidine. The new stands of DNA form during the mitotic division, in which it is consolidated. The limit of cell division that has been occurred to the effect of a test agent is reflected to radioactivity of DNA obtained from assay which can be measure by the used of commercially available scintillation kit.\[^{10}\] The advantage of these incorporation assays is related to the direct measures of proliferation. Other Indirect methods those measure mitochondrial activity (e.g. MTT assay) require additional confirmation, since test agents could affect the assay processing without affecting cell viability.

The overall performance of the assay started from addition of lymphocytes for few days with the provocative chemical compound which is related to growth factor (3H-Thymidine). The 3H-Thymidine get incorporates into the newly form DNA which is to be synthesized, when cell get proliferate. TCA-precipitation may be used historically for the separation of cell component. This separation help us in washing of incorporated 3H-Thymidine, then proceed towards the reaction(solubilisation) with 1M NAOH and make it neutral with the addition of HCL(neutralization).\[^{10}\] Then remaining sample was shifted towards scintillation vial for quantification. Commercially available kits (Microbeta), makes it possible to use cell harvester and 96-format glass-fiber filtermats. Other cell component and specifically newly synthesis DNA which is to be process get incorporated into In the harvesting step , which is get conquer into the filtermat, in which buffer will be using as a washing solvent. Before reaching to the the harvesting step the cell which get adhere, required a trypsin to slacken them. Before counting in the microbeta the, solution get dried and scintillation cocktail is adapted.

5. **Alamar Blue Assay**: Resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) is a non-toxic, cell permeable agent that is blue in colour (blue dye), which itself weakly
fluorescent till the irreversible conversion into dark red colour fluorescent Resorufin.\cite{11} For different types of cell conditions, like bacterial and mammalian cell the alamar blue agent is used as Redox indicator. Commercially it is available as the sodium salt. The agent is termed under alamar blue which is also available under other name like vibrant and uptiblue. Alamar blue assay shw good co-operation to attributing viability Assays such as formazan related assays (MTT/XTT). It also can be multiplexed with several chemiluminescent assays.

To study the proliferation of various cell like human or animal quantitatively by the used of this assay. This assay may also be used to determine the relative the cytotoxicity of various chemical compound within various chemical classes.\cite{10} The indicator is water soluble therefore assay is simple to perform, thus eliminating the washing/fixing or extraction process. the proliferation of various cell lines can be measure by the use of alamar blue assay, the indicator behave as health indicator for cell an dused there reducing power for quantification. which allowing to establish relative cytotoxicity of agents within various chemical classes. They prolong the reducing enviornment in the cytosol of the cell when they are alive. Once the indicator get enter into the cell entering the cells, it get reduced to resorufin, which is highly florescent and red in colour.\cite{10,11} Viable cell will increase the colour intensity of the media containing the cell by making it highly fluorescence.

Specifically, both florescense and change of colour occur due the incorporation of the oxidation-reduction (REDOX) indicator (Resazurin) that cause reduction of the medium chemically arise from from cell growth. The precise oxidation-reduction indicator applied into assay has been carefully preferred because of different characteristic. It will exhibit minimum toxicity to the cells.\cite{12} It could yield a luminous, stable specific change which is fluent to interpret. They are following, the indicator shows colorimetric effect in the relavant range related to cellular metabolism cause by reduction. When cell get ready to grow, then metabolic activity cause a chemical reduction of the indicator. The relieve growth posses a reduced environment in the cell while growth inhibition maintains an oxidized environment. The changes of redox indicator by showing florescent and changing colour is relavant to the growth of the cell.\cite{11,12}

6. LDH assay: There are two type of cell death programe which is percieved in both disease pathologies and normal, one is apoptosis and other one is necrosis. The necrosis cell death depend on the permeabilization of plasma membrane. Because of this permeabilization, the inflammatory responses may come which may be reflected towards the inflammatory
diseases.\textsuperscript{[13]} Death cytokines in the TNF (tumor necrosis factor) family are potent triggers of necrosis. As compared to apoptosis, necrosis cell death was once pretend as an inert and accidental form of cell death. The damage to the plasma membrane will indicate the necrosis of cell or necrotic cell death. The enzyme such as alkaline and acid phosphatase, glutamate pyruvate transaminase, glutamate oxalacetate transaminase, etc. leads to the release during Cell death by apoptosis. The amplification of kinetic assays required to measured most enzyme activity, because of low amount of enzymes present in the many cells and, whereas LDH is a stable cytoplasmic enzyme which is released into the medium in a adequate amount by following loss of membrane integrity on the conclusion of apoptosis.\textsuperscript{[11,12]} Therefore LDH assay can be used to determine the effect of cytotoxic agent or any chemical compound and I has used to evaluate the cell membrane purity.

![Diagram](https://example.com/diagram.png)

**Figure. 3.**

Different chemical compound such as Anti-cancer drug damage the disease effectcd cell and cause release of LDH (cytoplasmic) which are extracted. The secreted LDH is allowed to react with the solution containing lactate, NAD+ and a colouring agent or dye.\textsuperscript{[13]} LDH act as an enzyme and catalyzes the reaction of conversion of lactate to pyruvate by reducing NAD+ to NADH. Calculating the amount of LDH release during the reaction help us to study necrosis. A tetrazolium salt can used in this assay to detect the leakage of LDH into culture medium. The reaction of conversion of lactate to pyruvate and released of LDH is goes on several step, first LDH yeild reduced nicotinamide adenine Di-nucleotide (NADH), which catalyzes the reaction of oxidation of lactate to pyruvate. In the next step, a tetrazolium salt which we added in the beginning of the reaction, used this freshly prepared NADH for the formation of colour compound called formazan in the presence of electron acceptor. Colorimetrically we can quantified the formation of formazan product at wavelength of 490-529nm. The amount of formation of colour compound formazan is propotional to amount of cell lysed.\textsuperscript{[14]} The percentage of necrotic cells present in the cell sample can be evaluate by taking linearity of the assay. Different types of commercial kits are available for performing different type of conditions.
7. Comet Assay: This assay is also known as Single cell gel electrophoresis (SCGE), which can measure the DNA damage in the eukaryotic cell. This is the technique, which help the researchers for the direct visualization of DNA damage in individual cell. The principle of comet assay, is that unfragmented DNA maintains well organise structure in the nucleus and distrubted when cell is damage.\cite{15} It detect the both single strand and double strand DNA and has a simple and inexpensive setup. Comet assay is therefore beneficial technique for predicting a responces to the drug that are effected by DNA structure. An adequate amount of irradiated cells were incorporated or suspended in the thin agarose gel, which is kept on a slide which were electrophoresed, and after that they get stained with a fluorescent dye to DNA binding. The current pulled the charged DNA get pulled by current from the nucleus such that broken DNA fragments get migrate further separate from charged fragment. The following images are formed due to the migration, which were subsequently named as 'comets', as per there appearance, which were measured or evaluate to determine the extent of DNA damage. the comet assay provides us direct or visual determination of the of DNA damage in the cells, bye this evaluation, it can be possible to measured the degree of damage cell from the population.\cite{15}

![Figure 3.](image)

During the treatment the response given by the cell can be different this will aid in the prediction of tumor response to specific treatment mechanism, by this principle the researcher can find out the amount of cell which may be resistant.\cite{14} The valuable mechanism of genotoxicity and DNA repair can be examine by this technique. Under variety of experimental conditions, the damage and repair of DNA can be go through by the used of this assay. The most relevant cytotoxic lesion such as DNA inter-strand crosslinks are produced by many chemotherapeutic agents such as the alkylating agents. The separation of interstrand DNA crosslinking are based methods like Chromatographic and electrophoretic methods are based on the principle that different types of lesions prevent denaturation of DNA (by heating or addition of alkali).\cite{16}
8. DNA fragmentation assay

Different types of anti-cancer drugs which had been used shown to induce apoptosis in susceptible cells, which is included in caspase mediated cell death. Various Biochemical events lead to change in the characteristic of the cell (morphology) and cause death. These changes include loss of cell membrane asymmetry and attachment, nuclear and chromosomal DNA fragmentation, chromatin condensation, etc. One of the most important steps in apoptosis is DNA fragmentation, a process which result leads into degrade DNA endonucleases during the apoptotic program by the activation of magnesium and calcium dependent nuclease. These enzyme degrade the higher order chromatin structure into smaller DNA piece(50bp in length) or into fragments (300 kb) subsequently. Extreme apoptosis causes atrophy, also called as ischemic damage, where as an inadequacy cause uncontrolled cell propagation or proliferation, which lead to cancer.

Basic fundamental of this Assay is depended on Terminal Deoxynucleotidyl Transferase (TDT) conciliated by Dutp nick-end labelling. The DNA strands of the cell get break into a large number of 3'-hydroxyl ends by the Anti-cancer drug or compound which is cytotoxic. The free 3'-hydroxyl ends of fragmented DNA get attach by TDT. Once it get incorporated into the DNA, the attach brDutp can be detected by anti-brDutp antibody using standard immuno-histochemical techniques or fluorescence microscopy.[17]

In a This assay is also called as nick translation because the DNA which is to be processed is treated with Dnase to produces single-stranded "nicks", This is then followed by replacement in nicked sites by DNA polymerase-I, which wil elongates the 3’ hydroxyl terminus, removing the nucleotides by 5'-3' exonuclease activity, replacing them with Dntps. The radioactive label DNA fragment use as a probe in the blotting procedures, which would act as an incorporated nucleotides provided in the reaction and it is radiolabeled in the alpha phosphate position. Similarly, a fluorophore can also be attached as a fluorescent labelling or an antigen for immunodetection.[15] When DNA polymerase I eventually detaches from the DNA strand, it will leave another nick in the phosphate backbone.[17] The nick has "translated" some distance depending on the processivity of the polymerase. This nick could be seal by DNA-ligase, or its 3’ hydroxyl group could serve as the template for further DNA polymerase I activity. Proprietary enzyme are available commercially to perform all steps in the procedure single incubation.
9. Trypan blue exclusion assay
The most premative and common methods for determining the cell viability is the trypan blue (TB) exclusion assay. Trypan blue is a dye that is cell membrane impermeable (960 dalton) and they are able to enter into compromised cell membranes. Once the trypan blue get enter into the cell, it will binds to intracellular proteins thereby rendering the cells a bluish colour. The trypan blue exclusion assay allows for a direct identification and enumeration of live (unstained) or dead (blue) cells in a given population. Although TB has been used to determine cell viability for many years, It is considered to be carcinogenic and must be handled with care and disposed of properly.[18] Over time TB actually forms crystal of dye or aggregates of it, for this reason it is suggested that TB should pass through the filter using a 0.2 micron filter before performing assays.

Most reserchers have been already observed that TB viability identification in samples which contain less than 70% viable cell, showing higher measured cell viability when fluorescent based detection methods is compared to this. Various Data advise the samples with low viability may not be optimal for performing viability measurements with TB.[18] The assay system enforce as an reproducible and automatic determination of human or anima cell densities together with differentiation between live(viable) and dead cells.[16] It is suggested that TB exclusion assay is mostly used for purified/isolated cell samples with viabilities should be greater than 70% and cultured cell lines.

![Trypan Blue Exclusion Methods](image)

**Figure. 4.**

10. Potato disc tumor induction assay
Bioassay methods are using over the years, to determine the anti-tumor activity or tumor inhibition specificity of plant extracts. These bioassay methods have been already yielded important discoveries from plant origin or by plant extract including vincristine, the phodophylo toxin and etc. The potato disc tumor induction assay was set to be useful for evaluating unknown and novel anti-tumor properties. The potato disc induction assay is based
on Agrobacterium tumefaciens (Rhizobium radiobacter), the bacterium is used to infect or for the formation of tumor or crown gall in the plant. The mechanism to produce tumor in plant tissue or to make tumorogenic plant by A.tumefaciens is as similar to the animal which shows the validity of such bioassay for evaluation of anti-tumor activity. Different type of studies had been already find numerous and prospective areas of similarity in the mechanism bacteria pathogen of plant and humans.

A.tumenefaciens is a Gram-negative soil bacterium, which is rod shaped and virulent that is the causative agent for tumor induction or for crown gall disease. Crown gall is a tumefaction disease in which a mass or lump of tissue protruding from roots and stems of woody and herbaceous plant by which tumor is produced. The masses of tumor could be soft or hard, with or without adulterous affect on the plant. By the infection of plant septum with A.tumenefaciens, a tumor-causing plasmid which is also called Ti-plasmid, found in the bacterial DNA, which is incorporated or inject into the plant’s DNA. When tissue is damaged, it release phenol or other chemicals, Which will activate the Ti-plasmid. This segment cause plant cell to multiply or develop rapidly without going into the apoptosis, resulting in tumor formation which is similar in the histology to the human and animal cancer or related to nucleic acid contents etc.

The resulting tumor is able to proliferate and also susceptible to block the apoptosis like in animal cancer. It has been proven that ability of A.tumefaciens for the formation of crown gall on potato discs and subsequent growth of the tumor was in good correlation with compounds there extracts, which are statistically much more informative for in-vivo anti-leukemic activity. By this consequence, the potato disc tumor induction assay could be usfull as a inexpensive, reliable or rapid preclinical procedure for anti-tumor activity.
CONCLUSION
The official, well-established or Standardized in-vitro screening methods are suitable for experimental determination of new anti-cancer agents. It seems plausible to overcome the large quantity of potential drugs to a few or most promising agents for further clinical testing by using detail or expressive procedure, from in-vitro to in-vivo. The clinical specification for which the drug is aimed, needs to be considered in the experimental evaluation for curative and palliative or other modalities for the tumor entity. Pre-clinical screening or in-vitro assay is necessary to optimized the compounds for further procedure of development of new anti-cancer agent. The Number of assay technologies are available that used standard and well establish plate readers to determine the metabolic markers to evaluate the number of viables cells in culture. Each cell viability assay has its own set of advantages and disadvantages. In the scenario of target-oriented cancer therapeutics, screening of compounds are made to measure towards the desired mechanism of tumor inhibition. They required a prudent design and validation of compound. In the past decade the empirical screening procedure are designed to evaluate highly potent or cytotoxic agents which produced low selectivity and efficacy in solid tumors when clinically using it. However, empirical screening procedure combined with novel or new ethical knowledge, arising from genome and proteome research as well as bio-information technologies might be the most beneficial for the determination or designing of new anti-cancer agent.

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