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Drug Discovery Methods and Medicinal Plants for Anticancer Treatment: Scientific Validation

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ABSTRACT

The aim of this review to explore the various medicinal plants with reported anticancer activity and methods for the evaluation of those plants. Carcinogenesis is the process of development of cancer cells or tumors by the process of uncontrolled cell proliferation. Cancer is a complex process that involves various steps such as initiation, promotion, conversion, and progression. Various synthetic and semi-synthetic agents are available to treat the various kinds of cancers but they all have some serious kind of adverse effect and some of them are lethal to the patients. Hence more research is going to explore the effectiveness of herbal agents for cancer treatment. Different in-vitro and in-vivo pre-clinical methods are available to evaluate the potency of an herbal drug such as Trypan blue dye exclusion assay, Lactic Dehydrogenase (LDH) assay, MTT assay, XTT assay, Sulforhodamine B assay, Induction of Ehrlich Ascites Carcinoma (EAC) Model.

INTRODUCTION

Cancer is a multi-step progressive group of disease that is characterized by the uncontrolled cell proliferation[1]. Cancer is a leading cause of morbidity and mortality in industrialized countries. Approximate 11 million people are diagnosed with cancer every year and it will be 16 million new cases every year by 2020. Cancer is also known as Malignant or neoplasm, a tumor is categorized as benign and malignant. A benign tumor does not invade nearby tissue or spread to another part of the body and is generally identified by the suffix "–oma" for example- adenoma, papilloma, fibroma, Hemangiomas, lipomas, myomas. A malignant tumor is made of cancer cells and it can spread to the nearby area of the affecting part of the body[1,4]. When the cancer cell moves to the bloodstream and lymph nodes and spread to any tissue is known as metastasis. Normally when the cell becomes old or damaged it may undergo apoptosis and new cell replace it but in the cancer cell, this normal process is disrupted and the old cell did not die and become a cancer cell. Cancer cell (tumor cell) ignores the apoptosis signal and divide uncontrolled. For the effective cure of cancer, it is necessary to detect or diagnose in early stage[3] (Table 1).

Table No. 1: Screening Guidelines for Early Detection of Cancer in Asymptomatic People[3]

Sr. No.	Type of Cancer	Test or Procedure
1	Breast Cancer	Breast self-examination Mammography
2	Colon and rectal cancer	Fecal occult blood test (FOBT) or fecal immunochemical test (FIT) Double-contrast barium enema
3	Prostate cancer	Digital rectal exam and prostate-specific antigen (PSA) blood test
4	Cervical cancer	Pap test or liquid-based test
5	Endometrial cancer	Information on risks and symptoms

Carcinogens are the substance that causes cancer it includes chemical, physical and biological agents. Exposure to chemicals such as aniline dye (bladder cancer), benzene (leukemia), and some drugs such as alkylating agents, epipodophyllotoxins, immunosuppressive agents (lymphomas), estrogens and anthracyclines known to cause various cancers. Physical agents include ionization radiation and UV light (Fig.1). The Epstein-Barr-virus (Biological agent) is an important factor for the initiation of African Burkitt's lymphoma[2,3].

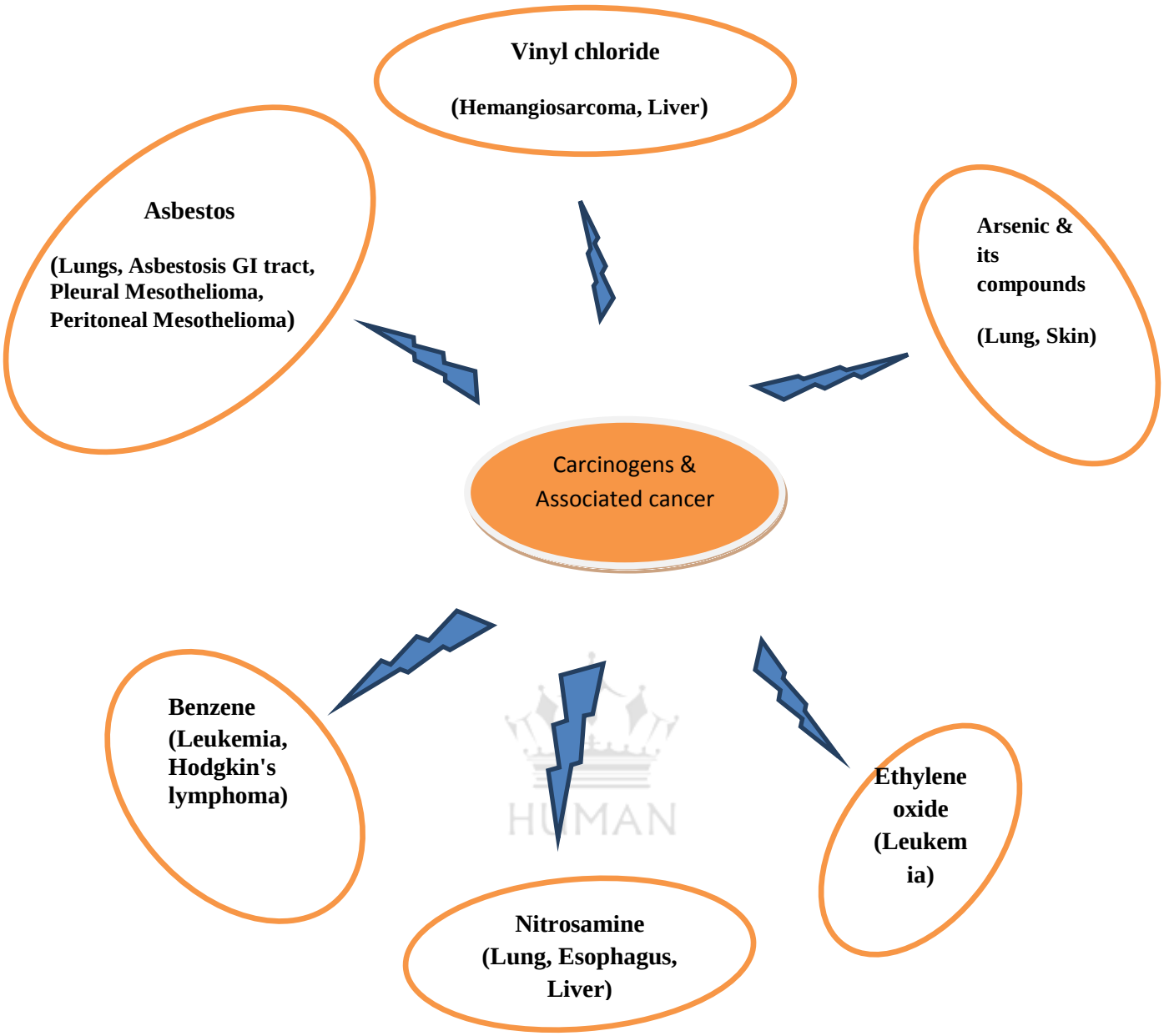

Figure No. 1: Carcinogens and associated cancer

Pathogenesis:

Carcinogenesis includes various steps such as initiation, promotion, conversion, and progression. Initiation requires exposure of the normal cell to a carcinogenic agent that produce genetic damage and irreversible cellular mutation. The second step of carcinogenesis is known as a promotion that provides a favorable environment for the growth of the mutated cell. This step is reversible as compared to the irreversible initiation step (Fig.2). If not detect clinically during 5-20years of life span mutated cell convert to cancerous cell (conversion or

transformation). The final stage or Progression involves further genetic changes, invasion of local tissue, and increased cell proliferation[4].

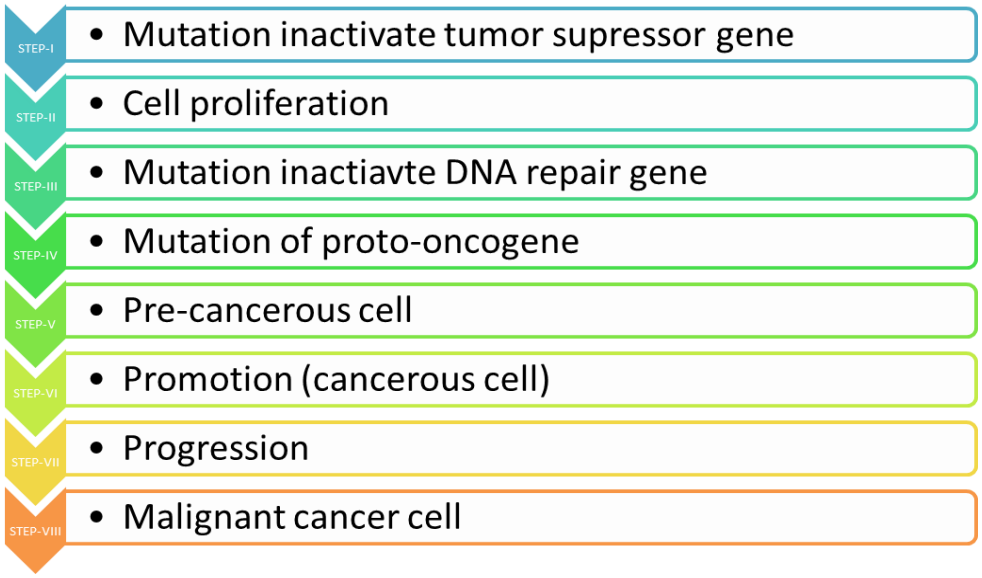

Figure No. 2: Different steps in cancer development

Oncogenes and tumor-suppressor are two major classes of genes involved in carcinogenesis. Oncogenes are developed from the proto-oncogenes. Proto-oncogenes play an important role in the regulation of normal cellular function including the cell cycle. Point mutation, chromosomal rearrangement, or gene amplification causes genetic alteration of proto-oncogenes and activate oncogenes. Activated oncogene produces an excessive amount of abnormal gene products that result in dysregulation of normal cell growth and proliferation. On the other hand tumor-suppressor gene inhibits irregular cellular growth and proliferation. Gene loss or mutation results in loss of control over the cell. The most common tumor suppressor genes are retinoblastoma and p53 genes. Mutation in p53 is associated with most types of cancer. DNA repair gene is another group of a gene that involved in carcinogenesis (Fig.3). Normally these gene repair damaged DNA if not repaired, they can activate oncogene and inactivate tumor-suppressor gene[3,4,5].

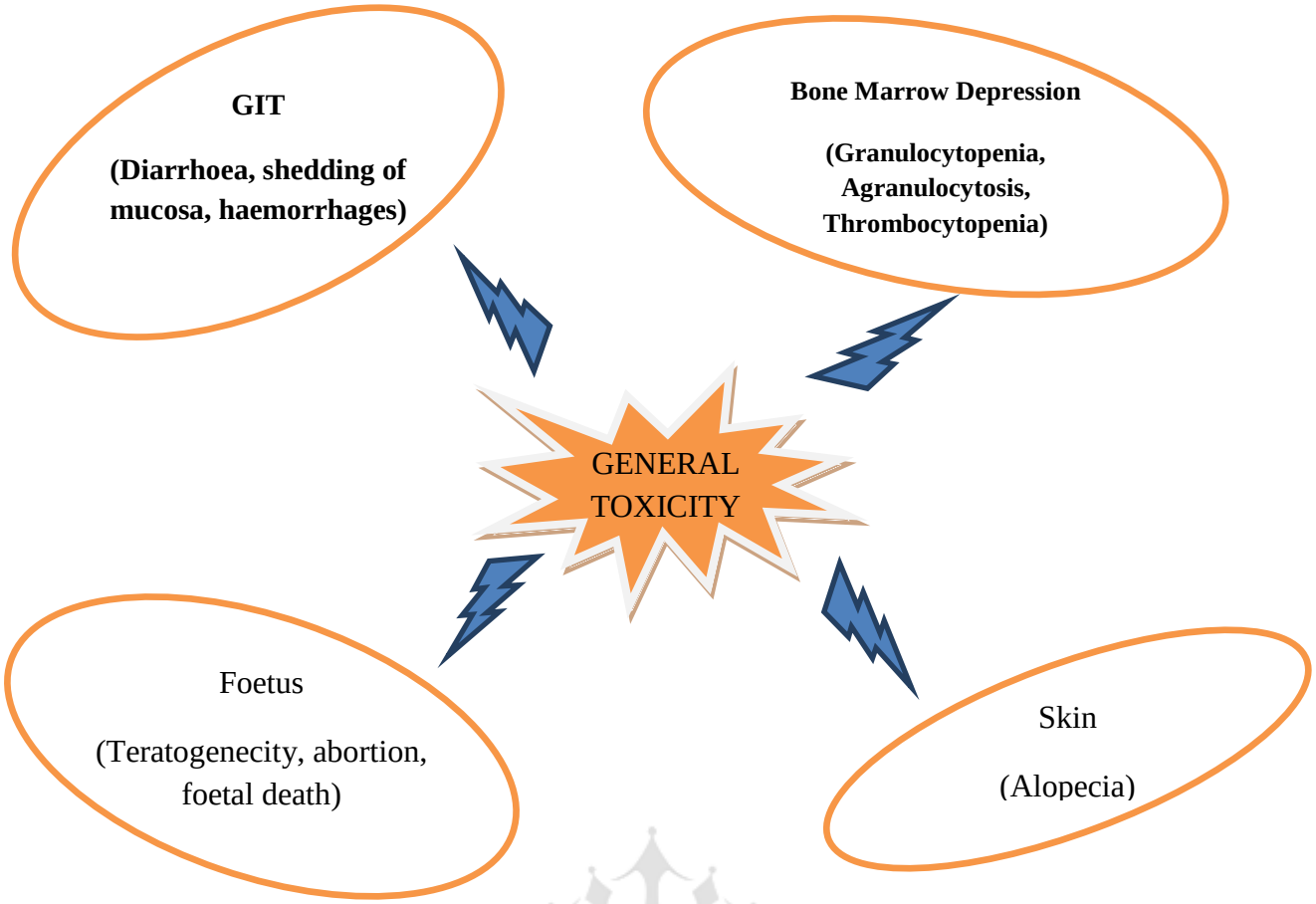

Figure No. 3: General toxicities of anti-cancer drugs

Stages of cancer development:

Initiation: The process of initiation in cancer development includes the establishment of stable and mutated cell that is irreversible. The carcinogens of this stage are known as initiators. These initiators bind with DNA and form an adduct that can lead to mutation and deletion that ultimately result in inappropriate DNA base pairing and DNA damage. Initiated cells lead to quiescent nondividing cells than induction of apoptosis and finally proliferation.

Promotion: The promotion is the second phase of carcinogenesis that involves the duplication of initiate cells and forms a genetically similar cell known as a preneoplastic or precancerous cell. In the promotion process, the cell number is increasing by the increased cell proliferation and/or decrease in the apoptosis process. This process involves repeated exposure to carcinogens.

Progression: This process includes the conversion of benign preneoplastic tumors or precancerous cells to malignant neoplastic tumors or cancer cells. Cell proliferation increases

DNA synthesis that ultimately leads to more DNA damage, chromosomal aberration, and translocations.

Diagnosis:

For tissue diagnosis, the tumor should be staged to determine the extent of cancer. The most common staging system for solid tumors is TNM (T= tumor, N=node, M=metastases), each letter is assigned with a numerical value that indicates the size or extent of cancer[3,4] (Table 2).

Table No. 2: TNM Staging Classification System for Cancer[3].

Sr. No.	Stage	T	N	M
1	Stage 0	Tis	No	Mo
2	Stage I	T1 T2	No No	Mo Mo
3	Stage IIA	T3	No	Mo
4	Stage IIB	T4	No	Mo
5	Stage IIIA	T1-2	N1	Mo
6	Stage IIIB	T3-4	N1	Mo
7	Stage IIIC	Any T	N2	Mo
8	Stage IV	Any T	Any N	Mo

Primary Tumor (T):

Tx: Primary tumor cannot be assessed

To: No Evidence of primary tumor

Tis: Carcinoma in situ: intraepithelial or invasion of lamina propria

T1: Tumor invades submucosa

T2: Tumor invades muscularis propria

T3: Tumor invades through the muscularis propria to subserosa

T4: Tumor perforates the visceral peritoneum and/or directly invades the other organs

Regional Lymph Nodes (N):

Nx: Regional lymph nodes cannot be assessed

No: No regional lymph node metastasis

N1: Perirectal lymph nodes

N2: Perirectal lymph nodes

Distant Metastasis (M):

Mx: Presence of distant metastasis cannot be assessed

Mo: No distant metastasis

M1: Distant metastasis

There are various synthetic drugs are available that cure cancer but they have so many adverse effects and toxicities (Table 3) and so more research is going on plant-derived or natural chemotherapeutic agents[5]. Folklorically Herbal medicines are used to cure various diseases (Table 4). Products from natural origin have been used to cure thousands of diseases. Traditionally plant drugs have been successfully preventing and suppressing various types of tumors.

Table No. 3: Therapeutic Agents for Treatment of Cancer and their Adverse Effects[5]

Sr. No.	CLASSIFICATION	EXAMPLE OF CLASS	ADVERSE DRUG REACTION
1	Alkylating agents	1. Cyclophosphamide 2. Chlormbucil 3. Melphalan 4. Thio-tepa 5. Busulfan 6. Carmustine 7. Lomustine 8. Dacarbazine	Alopecia, Cystitis, Bone marrow depression, Hyperuricemia, Pulmonary fibrosis
2	Antimetabolites	1. Methotrexate 2. 6-mercaptopurine 3. 6-thioguanine 4. 5-fluorouracil 5. Cytarabine	Megaloblastic anemia, Pancytopenia, Bone marrow depression
3	Vinca alkaloids	1. Vincristine 2. Vinblastine	Peripheral neuropathy, Alopecia, Bone marrow depression
4	Taxanes	1. Paclitaxel 2. Docetaxel	Myelosuppression, Stocking and Glove neuropathy, Arthralgia, Neutropenia
5	Epipodophyllotoxin	1. Etoposide	Alopecia, Leucopenia
6	Camptothecin analogues	1. Topotecan 2. Irinotecan	Bone marrow depression, Neutropenia, Anorexia
7	Antibiotics	1. Actinomycin d 2. Doxorubicin 3. Bleomycin	Bone marrow depression, Erythema, Desquamation of skin
8	Miscellaneous	1. Hydroxyurea 2. Procarbazine 3. L-asparaginase 4. Cisplatin 5. Carboplatin 6. Imatinib	Myelosuppression, Leucopenia. Thrombocytopenia, Liver damage, Pancreatitis

Table No. 4: Medicinal plant reported to have anticancer activity on different cell lines.

Sr. No.	PLANT NAME	EXTRACT	CELL LINE	REFERENCES
1	*Cynodon dactylon*	Petroleum ether	HEP-2 LARYNGEAL, HELA CERVICAL, MCF-7 BREAST CANCER	6
2	*Bauhinia variegate*	Methanol	EAC- MOUSE	7
3	*Datura stramonium*	Methanol	MCF-7 BREST CANCER	8
4	*Tillandsia recurvata*	Chloroform	A375(HUMAN MELANOMA), MCF-7 (HUMAN BREAST), PC-3 (HUMAN PROSTATE CANCER)	9
5	*Leucas aspera*	Water, Methanol	HeLa	10
6	*Glinus lotoides*	N-hexane, Dichloromethane, Methanol, Water	CALU-3, CACO-2	11
7	*Withania somnifera*	Hydroalcoholic (1:1)	MCF-7, A549 (LUNG), PA-1 (OVARY)	12
8	*Aerva lanata*	Ethyl Acetate	MCF-7	13
9	*Tabernaemontana divaricata*	Hydroalcoholic	HeLa	14
10	*Parthenium hysterophorus linn*	Ethanolic	K562	15
11	*Oldenlandia corymbosa*	Ethanolic	K562	15
12	*Tecoma stans*	Methanol	A549 (LUNG CANCER)	16
13	*Andrographis paniculata nees*	Ethanol	HEP-2 (LARYNX CARCINOMA)	17
14	*Barleria grandiflora*	Alcoholic	A549, DLA TUMOR CELLS, VERO	18
15	*Morus nigra*	N-hexane, Aqueous-methanolic	HeLa	19
16	*Sansevieria liberica*	Hydroalcoholic	SARCOMA-180, L1210 LYMPHOID LEUKEMIA	20
17	*Catharanthus roseus*	Aqueous, Methanol	MCF-7	21
18	*Berberis aristata*	Methanolic	MCF-7	22
19	*Asystasia travancorica*	Ethanol	DALTON ASCITES LYMPHOMA (DAL)	23
20	*Andrographis*	Water, Ethanol,	IMR32, HT-	24

	paniculata nees	Acetone	29(HUMAN COLON)	
21	*Psidium guajava*	Methanol, Hexane, Chloroform	KBMS, SCC4, U266	25
22	*Hordeum vulgare*	Water and Juice	HT-29, A549	26
23	*Averrhoa bilimbi*	Methanolic	MCF-7	27
24	*Ceropegia pusilla*	Ethanolic	HeLa	28
25	*Canthium parviflorum lam*	Ethanolic	DLA, HeLa	29
26	*Aerva javanica*	Hexane, Chloroform, Ethyl acetate, Acetone, Methanol	MCF-7	30
27	*Leea indica*	Chloroform, Ethyl acetate, Methanol, Ethanol and Aqueous	DU-145, PC-3	31
28	*Punica granatum*	Ethanol, Aqueous	CACO, HepGII	32
29	*Theobroma cacao*	Methanolic	MCF-7	33
30	*Ficus krishnae*	Pet. Ether	MCF-7	34
31	*Simarouba glauca*	Chloroform, Ethyl acetate, Methanol, Ethanol and Aqueous	T-24 BLADDER CANCER	35
32	*Fagaropsis angolensis*	Water, Methanol	VERO, E6, HeP-2 (THROAT), CT-26-CL-25	36
33	*Syzygium alternifolium walp.*	Hexane	MCF-7, DU-145	37
34	*Argemone mexicana linn.*	Methanolic	MCF-7, HeLa	38
35	*Eugenia jambolana*	Acetone, Methanol, Ethanol	Hep-2	39
36	*Annona squamosa*	Ethanolic	MCF-7	40
37	*Euonymus europaeus l.*	Hydroalcoholic	HUMAN MELANOMA CELLS	41
38	*Zingiber officinale*	Methanolic, Ethyal acetate, Pet ether	HCT-116, SW480, LoVo cells	42
39	*Hibiscus rosa-sinensis*	Acetone	HeLa	43
40	*Aegle marmelos*	Ethanol	DLA	44
41	*Terminalia arjuna*	Pet ether	HEP-2, HT-29	45
42	*Tamarindus indica*	Ethanol	HT-29	46
43	*Cuminum cyminum linn*	Ethanol	COLON 502713, COLO-205, HEP-2, A-549, OVCAR-5, PC-5, SF-295	47
44	*Carica papaya*	Pet ether, Ethyl	TK-10, UACC-62,	48

		acetate, Methanol	MCF-7	
45	*Ocimum sanctum*	Ethanol	NCI-H460	49
46	*Swertia chirata*	Methanol	MDA-MB-231, MCF-7	50
47	*Momordica charantia*	Water	HCT 116, MCF-7, HepG2	51
48	*Allium sativum*	Hydro-alcoholic	MCF-7, A549, PA-1	52
49	*Thevetia peruviana*	Methanolic	HTB-38, HTB-177, HTB-81, HTB-22,	53
50	*Coriandrum sativum*	Ethanol	HT-29	54
51	*Nardostachys jatamansi*	Pet ether, Methanol, Diethyl ether, Ethyl acetate, Aqueous	MCF-7, MDA-MB-231	55
52	*Nerium oleander*	Methanolic	HT-144, MCF-7, MCI-H460, SF-268	56
53	*Musa paradisiaca*	Ethanol	HeLa	57
54	*Echinacea purpurea*	Methanol	BT549	58
55	*Myristica fragrans*	Ethanol	COLON502713, COLO205, HEP-2, A-549, OVCAR-5, PC-5	59
56	*Aloe vera*	Lyophilized	HEPG2	60
57	*Foeniculum vulgare*	Methanol	MCF-7, HEPG-2, HCT 116,	61
58	*Citrullus colocynthis*	Hydroalcoholic	MCF-7, AGS	62
59	*Chlorophytum tuberosum*	Pet ether, Chloroform, Ethanol, Water	MCF-7, HELA, COLO-205, HEPG2, HL-60	63
60	*Solanum nigrum*	Methanol, Chloroform	PC-3, HELA	64
61	*Piper nigrum*	Ethanol	HCT-116, HCT-15, HT29	65
62	*Withania somnifera*	Ethanol	PC-3, DU-145, HCT-15, A549, IMR-32	66
63	*Tribulus terrestris*	Ethanol	MCF-7	67

Classification of carcinogens:

Carcinogens are classified according to different governmental and non-governmental agencies which are as follows (Fig.4):

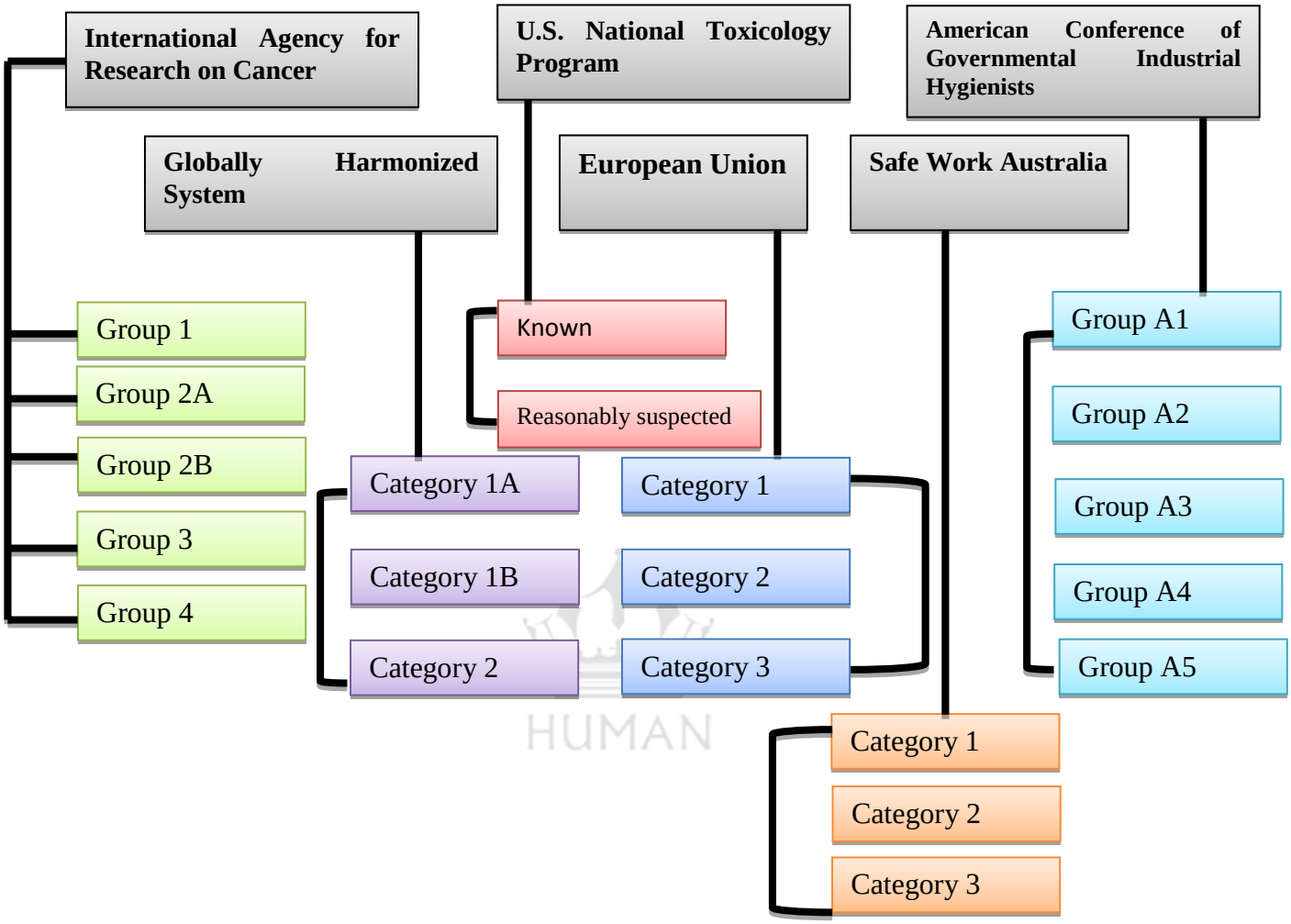

Figure No. 4: Classification of carcinogens

International Agency for Research on Cancer (IARC):

IARC is a part of the UN World Health Organization that is established in 1965. It published a series of Monographs on the Evaluation of carcinogenic Risks to Humans. According to IARC, the carcinogens are grouped in five classes.

Group 1: The agent or mixture is carcinogenic to humans. The exposure circumstance entails exposures that are carcinogenic to humans.

Group 2A: The agent or mixture is probably carcinogenic to humans.

Group 2B: The agent or mixture is possibly carcinogenic to humans.

Group 3: The agent or mixture or exposure circumstance is not classifiable as to its carcinogenicity to humans.

Group 4: The agent or mixture is probably not carcinogenic to humans.

Globally Harmonized System (GHS):

GHS is a UN initiative of assessing the different chemical risk that exists around the world. According to GHS, the carcinogens are divided into three categories.

Category 1A: The assessment is based primarily on human evidence.

Category 1B: The assessment is based primarily on animal evidence.

Category 2: Suspected human carcinogens

U.S. National Toxicology Program:

A biennial Report on Carcinogens is produced by the National Toxicology Program of the US department of health and human services. It classifies carcinogens into two groups:

Known to be a human carcinogen.

Reasonably anticipated being a human carcinogen.

American Conference of Governmental Industrial Hygienists (ACGIH):

ACGIH published the threshold limit values for occupational exposure and monograph on workplace chemical hazards.

Group A1: Confirmed human carcinogen

Group A2: Suspected human carcinogen

Group A3: Confirmed animal carcinogen with unknown relevance to humans

Group A4: Not classifiable as a human carcinogen

Group A5: Not suspected as a human carcinogen

European Union (EU):

It consists of three categories:

Category 1: the substance is known to be carcinogenic to humans.

Category 2: substances that should be regarded as if they are carcinogenic to humans.

Category 3: substances which cause concern for humans, owing to possible carcinogenic effects but in respect of which the available information is not adequate for making a satisfactory assessment.

Safe Work Australia:

Safe work Australia published the approved criteria for classifying hazardous substances. This consists of three categories:

Category 1: Substances are known to be carcinogenic to humans.

Category 2: Substances that should be regarded as if they were carcinogenic to humans.

Category 3: Substances that have possible carcinogenic effects in humans but about which there is insufficient information to make an assessment.

Screening Methods of anticancer activity:

The various pre-clinical screening methods have been developed to measure the effectiveness of natural as well as synthetic anticancer agents (Fig.5). Large scale screening using animals are used in the past is highly unethical and must be strictly regulated. The committee for control and supervision of experiments on animals (CPCSEA) in India regulate the screening using animals[1]. The in-vitro method includes various kind of human cell lines (Table V) that used during Trypan blue dye exclusion assay, Lactic Dehydrogenase (LDH) assay, MTT assay, XTT assay, Sulforhodamine B assay, and in-vivo method includes induction of Ehrlich ascites carcinoma, Tumor Xenograft model and Genetically Engineered Mouse (GEM) Model[2].

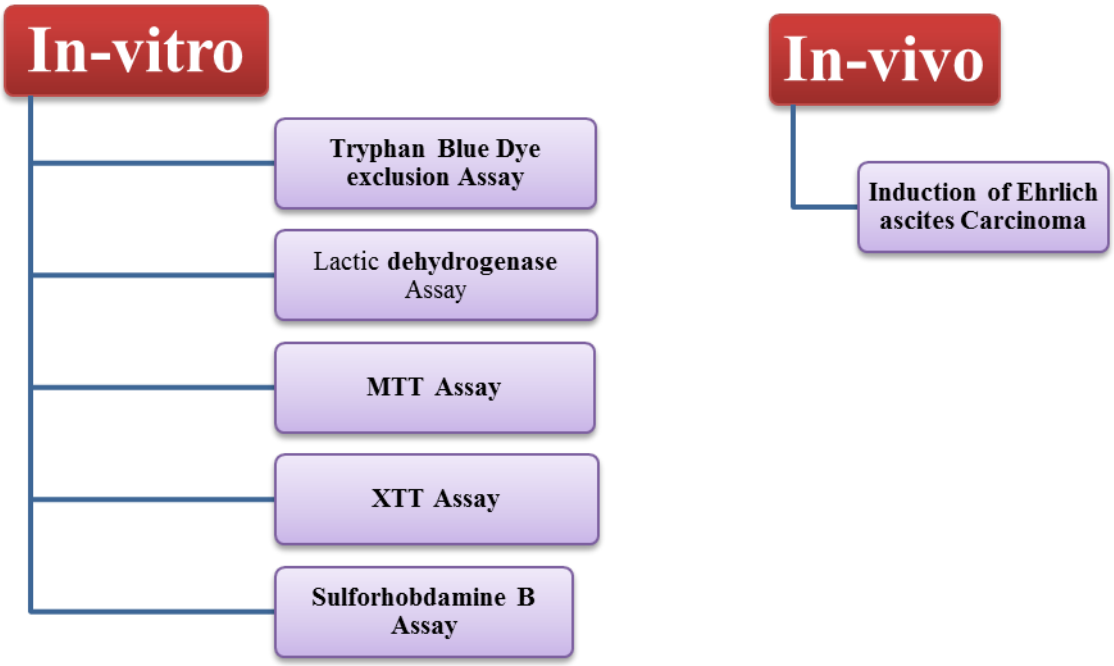

Figure No. 5: Classification of screening methods of anticancer activity

Table No. 5: Cell Lines for the screening of anticancer activity[2].

Sr. No.	Cell line	Types of Cancer
1	HCT 116	Human Colon Cancer
2	MCF-7	Human breast cancer
3	PC-3	Human prostate cancer
4	HT-29	Human colon
5	HELA	Cervical Cancer
6	HEPG-2	Human Liver Cancer
7	OVCAR-5	Human Epithelial Carcinoma
8	A-375	Human melanoma
9	CALU-3	Human Lung Cancer
10	CACO-2	Epithelial Colorectal Adenocarcinoma
11	PA-1	Ovary cancer
12	K562	Leukemia
13	DLA	Dalton Lymphoma ascites
14	HEP-2	Larynx carcinoma/ throat
15	A-549	Lung cancer

In-Vitro Methods:

Tryphan Blue Dye exclusion assay[2,4]:

In this assay, the cell line washed with Hank's Buffered Salt Solution (HBSS) and centrifuged for 10-15 minutes at 10,000 rpm, and this procedure is repeated three times. The centrifuged cells are suspended in a known quantity of Hank's Buffered salt solution and the cell count is adjusted to 2×10^6 cells/ml. The cell suspension is distributed into Eppendorf tubes (each 0.1ml containing 2 lakhs cells). The cells are exposed to test drug and incubated at 37ºC for 3h. After 3h equal quantity of the drug-treated cell is treated with tryphan blue (0.4%) and leave for 1 minute. Viable (No color) and non-viable (Coloured) cell count is recorded within 2 minutes with the help of a hemocytometer (Fig.6). If cells are kept for a long period than living cells also take up the colour. The percentage of growth inhibition is calculated by the following formula:

Growth Inhibition (%) = 100- (Total Cells-Dead Cells)/ Total Cells × 100

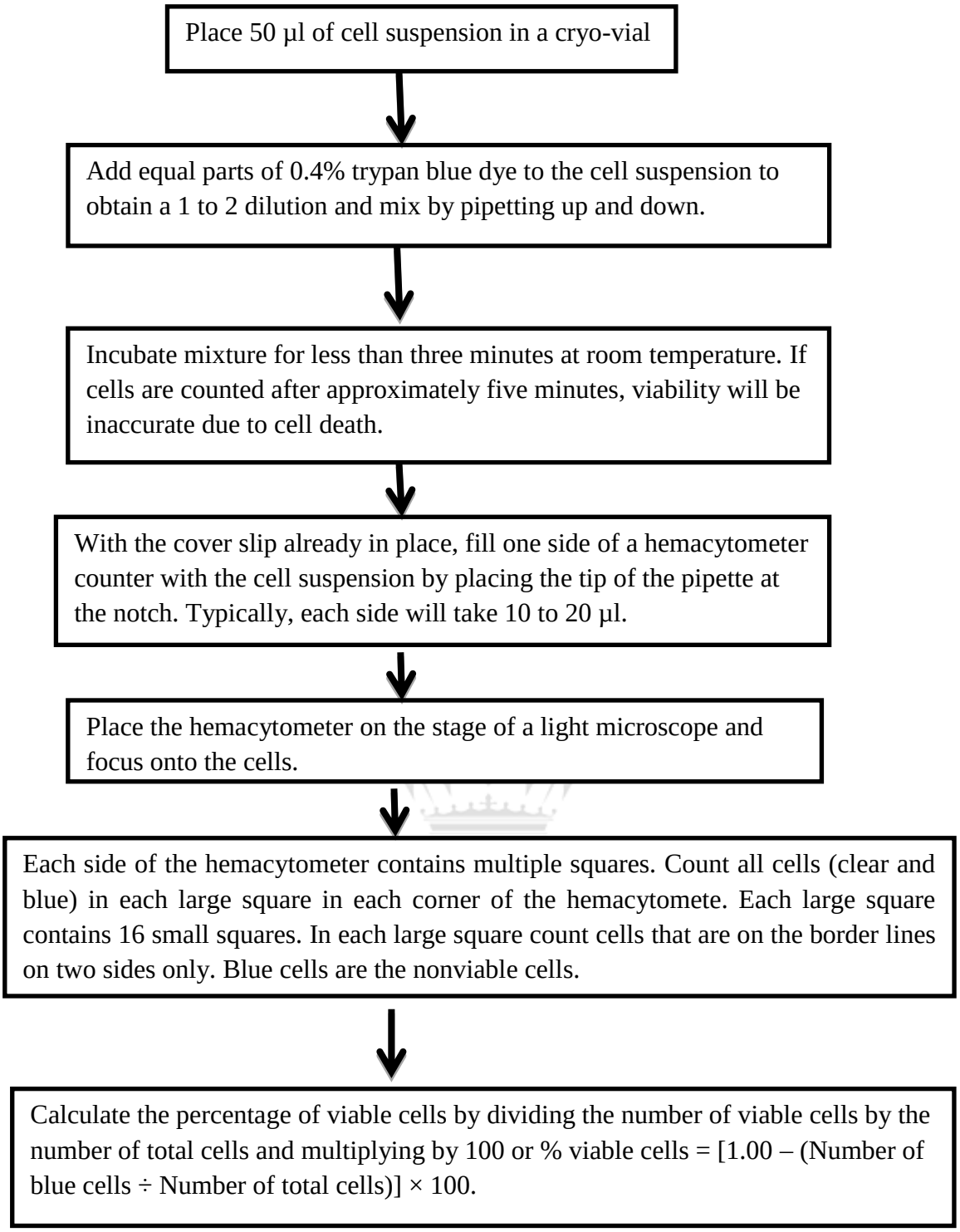

Figure No. 6: Flow diagram of Trypan Blue Dye Assay

Lactic Dehydrogenase (LDH) Assay[2,25]:

LDH (Lactic dehydrogenase) Activity is measured spectrophotometrically in the culture medium at 340nm by analyzing NADH reduction during the pyruvate-lactate transformation. Cell line are lysed with 50mM Tris-HCL buffer (pH 7.4) + 20mM EDTA+ 0.5% Sodium Dodecyl Sulfate (SDS), cells are further disrupted by sonicator and centrifuged at 13000rpm

for 15 minutes. 1ml of final assay mixture for the enzymatic analysis consists of 33µl of the test sample in 48mM PBS (pH 7.5) + 1mM Pyruvate and 0.2 mM NADH. The percentage of LDH released is calculated as a percentage of the total amount of enzymatic activity.

MTT Assay[2,29]:

This method is based on the ability of viable cells to metabolize yellow tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to purple formazan crystals by mitochondrial dehydrogenases. The cells are seeded in 96-well plates with a density of 104cells/well and incubated for 24h at 37C and 5% CO2. The cells are treated with different concentrations of the sample. After 12, 24, and 48 h treatment 10µl of MTT labeling reagent is added to each well. Then plates are incubated at 37C and 5% CO2 for 4h. Then 100 µl of solubilization solution is added to each well and incubate at 37C to dissolve formazan crystals. Finally, absorbance is taken by ELISA plate reader at a wavelength of 570nm. The percentage of cytotoxicity and cell viability was calculated using the following formula:

Percentage cytotoxicity =1- [mean absorbance of treated cells/mean absorbance of negative control] as percentage viability.

XTT Assay[2,4,39]:

The tetrazolium salt (2,3-bis[2-Methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt (XTT) is specially used to quantify viable cells. The assay is designed for quantification of cell growth and viability spectrophotometrically without the use of radioactive isotopes and is based on the conversion of yellow tetrazolium salt, XTT, to form an orange formazan dye by mitochondrial dehydrogenase, occurs exclusively in living cells. Cells are grown in growth medium +10%FBS in 96-well plates until 70-80% confluence. This solution is then treated with a test sample for 24h. 50ml of XTT is added to each well and cells are incubated at 37C for 4h. The formazan dye formed is soluble in aqueous solution and optical density is taken at 450nm and compared with control wells with a screening multiwall spectrophotometer ELISA reader. The reference wavelength is 650nm.

Sulforhodamine B Assay[15]:

Sulforhodamine B assay is a bright pink aminoxanthene dye that binds to basic amino acids in low acidic conditions and dissociates under basic conditions. Cell lines are placed in a 96-

well plate at 5000-10000 cells/well. Cells are allowed to adhere to wells wall and then samples are added to triplicate in serial 3-folds dilutions. Each well is diluted with water at 1:10 dilution. Then plates are incubated at 37C, 5% CO2 for 3 days than assayed for growth inhibition using sulforhodamine B (SRB) assay. Cells are fixed by the addition of cold 50% trichloroacetate acid to a final concentration of 10%. Cells are washed five times with deionized water. The cells are then stained with 0.4% SRB dissolved in 1% acetic acid for 15-30 min. and subsequently washed 5 times with 1% acetic acid to remove unbound strain. Then plates are analyzed on a microplate reader at 595nm.

Percentage growth inhibition = [control-sample]/ control × 100

***In-vivo* models:**

Induction of Ehrlich ascites carcinoma:

Anticancer activity of the sample is determined using Ehrlich Ascites Carcinoma (EAC) tumor model in mice. The Ascitic carcinoma tumor is transplanted in mice and after 15 days the animals are divided into groups of 12 animals each. The ascitic fluid is drawn using an 18G needle and then the ascitic fluid is diluted in normal saline to get a concentration of 10^6cells/ml. This suspension is injected Intraperitoneally to obtain ascitic tumors. The mice are weighed on the day of tumor inoculation and then once in 3 days thereafter. The standard first dose is injected on the tenth day and last dose on the fifth day intraperitoneally. After the last dose administration, six mice from each group are sacrificed for antitumor and hematological study parameters. The remaining animals in each group are kept to check the mean survival time (MST) of the tumor-bearing animals[2,23].

Most common types of cancer and research conducted for their treatment:

Lung Cancer[68]:

Pulmonary carcinoma or lung cancer is the most common cancer in the world. The risk factor for lung cancer includes tobacco, smoking, exposure to carcinogens such as radon, formaldehyde, acrylonitrile, cadmium, ethylene oxide, and isoprene. These compounds cause DNA damage by forming the adducts with DNA or can cause replication error. This type of error in replication or repair can result in mutation in tumor suppressor genes leading to

cancer. Various researches on different plants and plant product were conducted to evaluate the anti lung cancer activity, some areas:

The antimetastatic effect of aqueous and methanolic extract of *Phyllanthus spp*. On human lung (A-549) and breast (MCF-7), cancer cell lines were established using MTS reduction assay by Lee *et.al*.in 2011. The result revealed that the presence of polyphenol compounds in Phyllanthus spp might be a valuable compound in the treatment of metastatic cancer[69].

The nanoparticles of ethanolic extract of *Selaginella doederleinii* leaf were evaluated for human lung cancer cells A-549 by Syaefudin *et.al*.in 2016. The result revealed that the concentration of 167µg/ml showed maximum inhibition of cancer cells[70].

The study was conducted by Mistry *et.al.* in 2018 to evaluate the antiproliferative effect of methanol extract of *Butea monosperma* leaf against superoxide scavenging assay, metal chelating assay, DPPH, and MTT assay by using A-549 human lung carcinoma cell and chick embryo fibroblasts as a control. The result revealed that the methanolic extract of *Butea monosperma* showed potential for tumor treatment[71].

The antioxidant and cytotoxic activity of *Tecoma stans* against lung cancer cell line A-549 was studied by Robinson *et.al*.in 2017 by using MTT assay. The result revealed that 99% inhibition of cells was showed on 100µg/ml concentration[72].

The study was conducted by Aydemir *et.al*.in 2015 to investigate the cytotoxic and apoptotic effects of *Ebenus boissieri* Barbey on human lung cancer cell line (A-549) using 3- (4,5-dimethyl thiazolyl-2)-2,5-diphenyltetrazolium bromide assay. The result indicates that the hydroalcoholic extract of *E. boissieri* has anti-apoptotic and anti-carcinogenic activity[73].

The study was conducted by Ranga *et.al.* in 2005 to investigate the anti-lung cancer activity of Indian traditional medicine Rasagenthi Lehyam by using A-549, H-460 cancer cell line, and one normal bronchial epithelial (BEAS-2B) cell line. The result of the study showed that chloroform fraction has significant inhibition activity of cell proliferation and induction of A-549 and H-460 cells but not the normal cells[74].

The study was conducted to evaluate the anticancer activity of *Limonia acidissima* and *Syzygium cumini* against the H-460 lung cancer cell line. The study was designed by Tripathy

*et.al.*in 2016. Result of the study showed that methanolic extract of *Limonia acidissima* and *Syzygium cumini* have significant inhibition of H-460 cell lines[75].

Breast cancer[76,77,78]:

Breast cancer is the second most common cancer that is associated with the persistently elevated blood levels of estrogen. The major estrogen is estradiol, can be metabolized to quinone derivatives that form adducts with DNA. These adducts can cause the removal of bases, depurination, inaccurate repair leading to mutation, and finally cancer. Estrogen produces carcinogenesis by three mechanisms: (1) the metabolism of estrogen to genotoxic carcinogens, (2) tissue growth stimulation, and (3) metabolism of ROS leading to increased oxidative DNA damage. There are various studies had been conducted to evaluate the anti-breast cancer activity of different plant extract, some are as follow:

The methanolic extract of fruit pulp of *Syzygium cumini* was evaluated for in vitro cytotoxicity against MCF-7 cells using MTT assay by Tripathy *et.al.*in 2015. The inhibition of cell viability was investigated at different concentration[79].

The aqueous and ethanolic extract of the whole plant of *Amaranthus tricolor* Linn was investigated on MCF-7 and MDA-MB-231 breast cancer cell line using MTT assay, neutral red assay and trypan blue assay methods by Kanbarkar *et.al.*in 2018. The result showed that the ethanolic and aqueous extract has significant activity against the MCF-7 and MDA-MB-231 breast cancer cell line[80].

The hexane leaf extract of *Acalypha indica* L. was conducted on MCF-7 cell lines by MTT assay method using cisplatin as positive control by Chekuri *et. al.* in 2017. Amongst the various concentrations, the 50µg/ml showed the maximum inhibition effect[81].

The aqueous extract of *Rosa beggeriana* Schrenk was conducted on LCLPI-11, MCF-7, and HSkMC using MTT, BrdU, and TUNEL assay by Zarei *et.al.*in 2019. The result showed that ethanolic extract was more potent than aqueous extract[82].

The anticancer activity of *Bauhinia rufescens* leaf extract was conducted on MCF-7 human breast cancer cell lines by Garbi *et.al.*in 2015. The concentration of 31.25µg/ml and above petroleum ether and 500µg/ml of methanolic extract was found to be cytotoxic in MCF-7 cell line[83].

The anticancer potential of hexane, chloroform, ethyl acetate, acetone, and methanol extract of leaf and leaf derived callus of *Aerva javanica* was studies against MCF-7 breast cancer cell line by Kamalanathan *et.al.*in 2018. The result showed the dose-dependent anti-proliferative activity[84].

The cytotoxic and apoptotic inducing activity of *Amoora rohituka* leaf extract was studied against the human breast cancer cells by Singh *et.al.*in 2018. The result revealed that extract showed anticancer activity with less toxicity[85].

Stomach Cancer:

Helicobacter pylori are the main causative agent for stomach cancer that accounting for approximately 9.7% death every year. Long term infection with H. pylori causes gastritis and production of reactive oxygen species (ROS) that result in oxidative damage in DNA. The altered DNA base pairing, replication error, and repair alteration leads to mutagenesis and ultimately stomach cancer[86, 87, 93, 94]. Various researches have been conducted for the treatment of stomach cancer, some are as follow:

The study was conducted by Ghazanfari *et.al.* in 2011 to evaluate the cytotoxic effect of Aloe vera, Ginger, Ziziphora, and saffron extract against AGS gastric cell line using MTT assay. The result showed that Ziziphora extract has a good candidate for AGS gastric cell line[88].

In vitro anti-proliferative and anti-invasive effect of polysaccharide-rich extract from *Trametes versicolor* and *Grifola frondosa* was investigated by Roca-Lema *et.al.* in 2019 against LoVo and HT-20 human colon cancer cells using MMP-2 enzyme activity. The result revealed that both the extract alone or in combination have significant anticancer activity[89].

The in-vitro and in-vivo activity of *Inula viscosa* was studied by Bar-Shalom *et.al.* in 2019 against colorectal cancer cells through induction of apoptosis against V/FITC/PI and TUNEL assay. The result showed that extract at a dose of 300µg/ml has significant activity against colorectal cancer cells[90].

In-vitro cytotoxicity activity of ethanolic extract of *Reissantia indica* was investigated by Sangeetha *et.al.* in 2019 against HT-29 colon cancer cell line using MTT assay. The result showed that extract has significant activity at 1000µg/ml concentration[91].

The antiproliferative and apoptosis induced potential of *Sesuvium portulacastrum* was evaluated by Chintalapani *et.al.* in 2019 against various cell lines using MTT assay. The result showed that plant extract has significant anticancer activity against HCT-116 cell line[92].

Colorectal cancer:

Colorectal cancer is the third most common cancer that occurs due to tobacco smoking and bile acid. Bile acid deoxycholic acid and/or lithocholic acid is the main component that causes the production of reactive oxygen species leading to DNA damage. Programmed cell death or apoptosis is also the main factor for causing the mutagenesis and cancer[95, 96, 97].

The study was conducted by Forouzesh *et.al.* in 2018 to evaluate the ethanolic extract of the cytotoxic effect of *Euphorbia tehranica* root against Caco-2 colorectal cancer cell line. The result revealed that plant extract has significant anticancer activity[98].

The cytotoxic effect of aqueous extract of *Portulaca oleracea* by Azarifar *et.al*. in 2018 against oral cancer cell line using MTT assay. The result showed that plant extract has significant anticancer activity[99].

The cytotoxic effect of *Caralluma fimbriata* was investigated by Ashwini *et.al*. in 2017 against the human colon cancer cell line using MTT assay. The result showed that plant extract has significant anticancer activity[100].

SUMMARY

This review explores the various medicinal plants that have reported in vitro anticancer activity on different cell lines such as MCF-7 (Human Breast Cancer), PC-3 (human Prostate Cancer), HT-29 (Colon Cancer), A-375 (Human Melanoma), PA-1 (Ovary Cancer), HEP-2 (Lyranx/Throat cancer), etc. by different methods such as Tryphan blue dye exclusion assay, Lactic Dehydrogenase (LDH) assay, MTT assay, XTT assay, Sulforhodamine B assay, Induction of Ehrlich Ascites Carcinoma (EAC) Model. There are still some researches that have to be explored to identify the active herbal agents with minimal adverse effect to save mankind from cancer.

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