Review Article

AN OVERVIEW FOR DETERMINING CYTOTOXIC ACTIVITY OF SOME INDIAN MEDICINAL PLANTS

V.V. Balaji, K. Pavani, S. Ahammad, S.Younus, P. Sailaja, J. Chandu, B. Pushpa Kumari*, M. Niranjan Babu
Department Of Pharmacology, Seven Hills College of Pharmacy, Tirupati, A.P., India – 517561
Corresponding Author Dr. B. Pushpa Kumari
Department of Pharmacology e-mail id: pushpakumari@shcptirupati.edu.in
Seven Hills College of Pharmacy Tirupati, A.P., India 517561

ABSTRACT
The aim of this review is to provide cell-based assays for the assessment of the cytotoxicity potential of some Indian medicinal plants against different cancer cell lines on Humans. The cytotoxicity of various cell lines such as HepG2, Hs578T, MCF-7, A549, SKOV3 were evaluated by performing various invitro anticancer assays such as MTT assay, MTS assay, SRB assay, HPLC method. All these plants have the potential for invitro studies and possess anticancer activity. The result of this present study confirmed that plant extracts have bioactive constituents with cytotoxic properties and which are useful for developing new anticancer drugs.

KEY WORDS
Cytotoxicity, Cancer cell lines (HepG2, MCF-7, SKOV3), Assays (MTT, MTS, SRB)

INTRODUCTION
Cancer is defined as the abnormal growth of cells in any part of the body that results in an organ bulge or a tumor of cells that is not beneficial to the body [Dorababu; 2016]. Due to the absence of widely available, comprehensive early detection techniques, the poor prognosis associated with late-stage diagnosis, and the diseases rising global occurrence [Sumitra; 2013].Cancer is a major global health cancer. Worldwide, cancer is a leading cause of both morbidity and mortality. The World Health Organization recently estimated that the annual cancer incidence and mortality rate in sub-Saharan Africa is 5,51,200 and 4,21,000 respectively per year. Approximately 70% of cancer- related deaths took place in low and middle income nations [Salwa; 2015]. Currently, the two biggest barriers to using chemotherapeutic drugs in the treatment of cancer are toxicity and tumor resistance [Ahmed; 2020].
Numerous pure chemicals have been investigated for their ability to combat cancer [Merajuddin; 2022]. Metabolites found in medicinal plants may be able to prevent the severe and often unbearable adverse effects associated with manufactured medications. While there are some novel methods in drug discovery, like combinatorial chemistry and computer based molecular modeling design, natural products remain crucial in the process of finding and developing new drugs[Salwa; 2015]. Since most known cancer medicines have side effects and different cancers respond differently to treatment, new approaches or substances must be found [Deniz; 2017]. Finding novel, safe therapy alternatives is seen as a difficult task [Ahmed; 2020]. Over the past century, technological advancements have allowed for the pure isolation of diverse plants active components for a variety of therapeutic uses [Merajuddin; 2022].

Since ancient times, Ayurveda, a traditional Indian medicinal system based on plant-based medicines, has effectively prevented or suppressed a variety of cancers using a variety of therapeutic modalities' [Sumitra; 2013]. The 1950s saw the initial recognition of the vital role that natural products played as anticancer agents, which paved the way for the development of several significant plant based anticancer therapies [Merajuddin; 2022]. In the majority of third world nations, herbal products continue to be the mainstay of healthcare [Deniz; 2017]. The importance of the synergistic action of composites, or mixtures of chemicals contained in the whole plant extract, is being highlighted by a growing number of researches [Merajuddin; 2022].
Based on the diversity of their chemical elements, including flavonoids, polyphenols, and alkaloids, plant extracts are rich in bioactive compounds that are important to the process of finding and developing new drugs [Ahmed; 2020]. Certain compounds found in plants, known as secondary metabolites, resemble toxins and have the potential to be hazardous to humans [Ahmed; 2020]. Typical commodities, particularly plants, have been utilized in the therapy for a range of illnesses for thousands of decades People from different ethnic groups live

in different parts of India, each with their own unique culture, customs, and medical expertise from ancient procedures. They use herbal medicine to treat a range of illnesses [Sumitra; 2013].

MATERIALS AND METHODS

A. Invitro methods

Many biological assays require the measurement of surviving and proliferating mammalian cells.

MTT (tetrazolium) Assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; was dissolved in PBS at 5 mg/ml and filtered to sterilize and remove a small amount of insoluble residue present in some batches of MTT. At the times indicated below, stock MTT solution (10µl per 100µl medium) was added to all wells of an assay, and plates were incubated at 37° C for 4hours. Acid-isopropanol (100µlof 0.04 N HCl in isopropanol) was added to all wells and mixed thoroughly to dissolve the dark blue crystals. After a few minutes at room temperature to ensure that all crystals were dissolved, the plates were read on Dyntech MR580 Microelisa reader, using a test wavelength of 570 nm, a reference wavelength of 630nm, and a calibration setting of 1.99 (or 1.00 if the samples were strongly coloured). Plates were normally read within 1 hour of adding the isopropanol.

LDH Assay

LDH assay is one of the colorimetric assays. Lactic Dehydrogenase activity is spectrophotometrically measured in the culture medium and in the cellular lysed with 50mM Tris-HCl buffer, pH 7.4 + 20 mM EDTA + 0.5% Sodium Dodecyl Sulfate (SDS), further disrupted by sonication and centrifuged at 13,000 X grams for 15 minutes. The assay mixture (1ml final volume) for the enzymatic analysis consists of 33 µl of sample I 48 mM PBS, pH 7.5 + 1 mM pyruvate and 0.2 mM NADH. The percentage of LDH released is calculated as percentage of the total amount, considered as the sum of the enzymatic activity present in the cellular lysate and that in the culture medium.

SRB Assay

Sulforhodamine B assay is a bright pink aminoxanthene dye that binds to basic amino acids in mild acidic conditions and dissociates under basic conditions. Cells are plated in 96-well flat bottom plates at 5000-10000 cell/well. The difference in cell numbers plated adjusts for differences in the growth rates of the various cell lines. Cells are allowed to adhere to the lwells overnight, then the samples are added to triplicate wells in serials 3-fold dilutions. Water is added to the control wells at 1:10 dilution in medium. These plates are incubated at 37° C, 5% CO_2 for 3 days, then assayed for growth inhibition using sulforhodmine B (SRB) assay. The cells are fixed by the addition of cold 50% trichloroacetic acid to a final concentration of 10%. After 1 hour incubation at 4°C, the wells are washed for 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 five times with 1% acetic acid to remove unbound stain. After the plates are air dried at room temperature, the bound dye is solubilized with 10 mm Tris base and the plates are analysed on a microplate reader (Molecular Devices) at 595 nm.

TLC Assay

Phytochemical screening by means of TLC was carried out for selected plants following the method of Wagner and Bladt. For this, dried extracts were reconstituted in ethanol to a concentration of 10mg/ml. 20µl of the extracts were spotted, in triplicate, on aluminium backed TLC plates chemical constituents were separated using any one of the three different eluent systems. For polar system, solvents used were ethyl acetate; for intermediate elution, solvents ethyl acetic acid/formic acid/glacial acetic acid were used; and for non-polar solvents were used. The TLC plates were dried under a stream of cold air until there was no solvent smell remaining to ensure complete removal of the eluting solvents. The plates were examined under UV light to detect coumarins that appear as blue, violet or yellow fluorescent spots. The specific groups present in the extracts were identified using specific developers. Vanillin sulphuric acid spray reagent were then sprayed on the dried plates and heated at 110°C for colour development for detecting the presence of monoterpene alcohol, bitter principle and saponin. Sprinkling the plates with 5% ethanolic solution of $AlCl_3$ resulting in the appearance of yellow or greenish fluorescent spot under UV light at 365 nm indicated the presence of flavonoids. A brown colorization reveals the presence of triterpenes and steroids. A 10% vanillin ethanol solution was used for detecting saponins, the presence of which results in blue, violet and yellow spots.

MTS Assay

MTS, a colorimetric assay, is very simple and widely used in response to compounds and agents from various sources to evaluate cell cytotoxicity and viability. Reduction of MTS tetrazolium compound or 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymeth-oxyphenyl)-2-(4-sulfophenyl)2H-tetraolium to soluble purple formazin dye in the presence of phenazine methosulfate, is done by NAD(P)H-dependent oxidoreductase enzymes in mitochondria of viable cells. In this assay, a group of tetrazolium reagents which include PMS have been lused to eliminate solubilization steps. These compounds can penetrate through the cell

membrane and convert tetrazolium to formazan product. To prepare MTS solution,dd 2 mg/ml of MTS powder to DPBS and dissolve it to have a clear yellow solution, then dissolve the 0.21mg/ml of polyethersulfone in MTS solution and add 1NHCl to adjust pH on 6.0-6.5 in the next step, filter the solution by filter of 0.2µm filter and transfer them into a sterile and light resistant container, the store MTS solution in light-protected place at -20℃ until analysis for immediate use store it at 4℃. To perform this assay, use seeded cell suspensions into 96 well plates in different tested groups which are incubated in a humidified atmosphere with 5% CO_2 at 37℃ in next step, add 20µl prepared MTS solution to each well and incubate for 1-4 hours at 37℃, and measure the absorbance by microplate reader at 570 nm.

BRINE SHRIMP Assay

Dried cysts of Artemisia salina were collected from an aquarium shop and hatched in artificial sea water for 48 hours to mature shrimp called nauplii. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. The test sample were prepared by dissolving them in DMSO plus sea water to attain concentrations of 10, 25,50,100,200,300,500 and 800 µg/ml. A vial containing 50µl DMSO diluted to 5ml was used as a control. Vincristine was used as positive control. After 24hours the number of survival of nauplii was counted and percentage of mortality was determined.

B. Cell Lines

PC-3 cell line was established in 1979 from bone metastasis of grade IV of prostate cancer in a 62 year old Caucasian male and these cells are influenced by epidermal growth factors. P53 is the most frequently mutated gene in cancer; it is mutated in some of aggressive cancers as small cell lung cancer, breast cancer. MCF-7 is a human breast cancer cell line with estrogen, progesterone receptors; it is derived from pleural effusion of 69 year old Caucasian. K562 cell line was the first human immortlised myelogenous leukemia cell line; it is a type of erythroleukemia and derived from 53 year old female chronic leukemia patient. HEK293 is derived from human embryonic kidney cells and these possess expression of membrane proteins. THP1 cell line is a human leukemia monocytic cell line used to study monocyte functions, nutrient and drug transport and it is isolated from peripheral blood of an acute monocytic leukemia patient.

MCF10 cell line is a non-tumorigenic epithelial cell line and it is a milk fat globule antigen and it is isolated from benign proliferative breast tissue. HepG2 cell line possess high proliferation rates and used in hepatotoxic studies and isolated from 15 year old liver tissue of causcasian male. HeLa cell line was first immortal human cell line; oldest and commonly used cervical cancer cell line. CHO-K1 cell line was derived from subclone from parenteral CHO cell line; an ovary of adult, female Chinese hamster. 3T3 cell line is a fibroblast cell line that was isolated from mouse embryo. EAC cell line is a udifferentiated carcinoma and one of the type of liver cancer. SK MEL2 cell line is commonly used human melanoma cancer cell line it induce mutations in BRAF and NRAS genes.SKOV3 cell line is a human ovarian cancer cell line with epithelial like morphology and are resistant to tumor necrosis factor. A549 cancer cell lines are lung carcinoma epithelial cells and used to model the alveolar type II pulmonary epithelium.

SW-620 cells were isolated from large intestine of 51 year old male colorectal cancer patient and used in cancer research. HGF-1 cell line was isolated from gingiva of 28 year old male patient. LNCaP cell line was isolated from left supraclavicular lymph node of 50 year old male metastatic prostate carcinoma patient. SW-48 cell lines were isolated from large intestine of 82 year old female colorectal cancer patient.EV-71 is a viral strain isolated from adult female oral cancer patient. Hs-578T cell line was isolated from 74 year old female breast cancer patient. HCT-116 cell line was isolated from colon of adult male with colon cancer and used to control PCR assay mutation in codon. AsPC-1 cell line was isolated from 62 year old female pancreatic cancer patient. CLL cell line is a type of B cells and it slowly affects the adults. 3T3L-1 cell line used to study cellular mechanisms of diabetes, obesity. T-47D cell line was isolated from 54 year old female breast cancer patient. HT-29 cell line was isolated from 44 year old colorectal adenocarcinoma patient.

Table1: A summary of Indian Medicinal plants having cytotoxicity against different cancer cell lines[10-49]

S. NO	PLANT NAME	SCIENTIFIC NAME	PLANT PART	MEDICINAL USES	CELL LINE	TYPE OF CANCER	TYPE OF ASSAY
1.	Neem	Azadirachta indica	Leaf	Leprosy, eye problems, skin diseases, septic sores	PC-3	Prostate cancer	MTT assay

				and infected burns			
2.	Tamarind	Tamarindus indica	Seed	constipation, liver and gallbladder problems and stomach disorders	RD Human lymphoma	Lymphomas	Brine shrimp assay
3.	Aloe Vera	Aloe vera	Leaf	Anti-inflammatory, antimicrobial Anti-aging and Heal wounds	P53	Liver cancer	Brine shrimp assay
4.	Henna	Lawsonia inermis	Leaf	Anti-inflammatory, anticancer, Analgesic and Reduces spams	MCF-7	Breast cancer	TLC assay
5.	Hibiscus	Hibiscus	Leaf, Stem	Antioxidant, antidiabetic, anticancer, antibacterial and Anti-inflammatory	K-562	Leukaemia	MTS, MTT assay
6.	Teak	Tectona grandis	Leaf, Bark	Used as laxative, sedative, treatment of piles, dysentery, leukoderma and anti-inflammatory	HEK-293	Kidney cancer	ABTS assay
7.	Curry leaves	Murraya koenigii	Leaf	Treatment of dysentery, antidiarrheal, antidiabetic, Treatment of morning sickness and nausea	Thp-1	Leukaemia	Colorimetric, MTT assay
8.	Sandal wood	Santalum album	Leaf	Anti-inflammatory, antiseptic, treatment of headache, stomachache, and urinary and genital disorders	MCF-7, MCF-10	Breast cancer	MTT assay
9.	Bael	Aegle marmelos	Leaf	Antidiarrheal, antidiabetic, treatment of dysentery and peptic ulcers	HepG2	Carcinoma	MTT assay
10.	Ant plant	Myrmecodia tubercosa	Leaf	Anticancer, antimicrobial,	HeLa	Cervical cancer	Brine shrimp assay

				anti-inflammatory, treatment of asthma and arthritis			
11.	Basil	Ocimum basilicum	Leaf	Used to treat stomach spasms, anti-inflammatory	CHO K1	Carcinoma	MTT assay
12.	Ivy gourd	Coccinia grandis	Roots	Antidiabetic, treatment of gonorrhoea	3T3 L1	B cell cancer	Brine shrimp
13.	Water lily	Water lillies	Leaf	Antidiabetic, anti-inflammatory, and used to treat liver disorders	MCF-7	Breast cancer	MTT assay
14.	Moringa	Moring oleifera	Leaf	Antioxidant, anti-inflammatory, anti-cholesterol	HeLa cell	Cervical cancer	MTT assay
15.	Amaranth	Amaranthus	Leaf	Antiseptic, anti-inflammatory, antifungal, anti-atherosclerotic	EAC	Breast cancer	DPPH assay
16.	Calotropis	Calotropis gigantean	Flowers	Anticancer, anti-inflammatory, antidiarrhoeal,	SK-MEL2	Melanoma	MTT assay
17.	Custard apple	Annona squamosal	Leaves	Anticancer, anti-inflammatory, antioxidant	MCF-7	Breast cancer	MTT & LDH assay
18.	Giloy	Tinospora cordifolia	Roots & Leaves	Anticancer, antiallergic, antidiabetic	AW13516	Oral cancer	MTT assay
19.	Peepal	Ficus religiosa	Bark, latex	Antidiabetic, antibacterial, anticancer, antioxidant	MCF-7, HCT-116	Breast cancer, colorectal cancer	MTT assay
20.	Betel	Piper betel	Leaves	Anticancer, antimicrobial, antidiabetic	HeLa	Cervical cancer	MTT assay
21.	Mango	Mangifera indica	Peel	Anti-ageing, anticancer, antidiabetic	HepG2, SW-620	Hepatic cancer, Colorectalcancer	MTT assay
22.	Periwinkle	Cantharanthus roseus	Stem	Antidiarrheal, anti-inflammatory, anticancer	THP-1	Human monocytic leukemia	Colorimetric XTT assay
23.	Vajradanti	Barleria prionitis	Leaves	Anti-rheumatic, anti-inflammatory	HGF	Human gingival fibroblast	MTT assay

24.	Sweet basil	Ocimum basilicum	Leaves	Used to treat stomach spasm and kidney diseases	HeLa, SKOV3	Cervical cancer, Ovarian cancer	MTT assay
25.	Guava	Psidium guajava	Leaf	Antidiarrheal, antidiabetic, and used to treat gastro intestinal infections	LNCaP	Prostate adenocarcinoma	MTT, SRB assay
26.	Touch me not	Mimosa pudica	Leaves, Roots	Antibacterial, antifertility, anticonvulsant, antidepressants	MCF-7, HepG-2	Breast cancer, Hepatoma	Brine shrimp assay
27.	Eucalyptus	Eucalyptus teriticornis	Leaves	Used to treat cough cold and bronchitis	SW48, HepG2	Colon cancer, Hepatic cancer	MTT assay
28.	Red silk cotton	Bombax ceiba	Flowers	Antidiarrheal, and used to treat male sexual disorders	A549, HepG2	Lung cancer, Hepatic cancer	MTT assay
29.	Banana	Musa acuminata	Leaves, Corms	Used to treat High Blood pressure	EV71, CHIKV	Enterovirus, Chickungunya virus	MTT assay
30.	Indian gooseberry	Phyllanthus Emblica	Fruit	Used to treat heartburn, antiaging, weight loss	HepG2, Hs578T	Hepatosarcoma, breast cancer	HPLC
31.	Indian Kudzu	Pueraria Tuberosa	Roots	Treat alcoholism, menopausal symptoms, fever	MCF-7, HepG-2, A-549, SKOV-3	Breast cancer, Hepatosrcoma, Ovarian cancer	HPLC
32.	Tridax Daisy	Tridax Procumbens	Leaves	Treat bronchial catarrh, diarrhea, dysentry	A-549, MCF-7	Lung cancer, Breast cancer	MTT assay
33.	Ajwan	Trachyspermum Ammi	Seeds	Relieve indigestion, bloating, treat ulcers	MCF-7, AsPC-1	Breast cancer, Pancreatic cancer	MTT assay
34.	Devil Trumpet	Datura Stramoium	Leaves	Treat stomach pain, fever, worm infestation	HepG-2	Liver cancer	MTT assay
35.	Night Jasmine	Nyctanthes Arbor	Flowers	Antioxidant, antbacterial, antfungal, anticancer, antiHIV	MCF-7, CLL	Breast cancer, Chronic Lymphocytic leukemia	Brine shrimp assay
36.	Rose	Rosa rubiginosa	Rose oil	Antidepressant, antispasmodic,	A-549	Liver cancer	MTT assay

				aphrodisiac,astringent			
37.	Banyan	Ficus Benghalensis	Powder	Burning sensation, ulcers, painful skin diseases, toothache	CHO, A-549	Ovary cell, Liver cancer	MTT assay
38.	Quail grass	Celosia Argentea	Flowers	Treat skin sore, eruption, heal burns, parasiticide	3T3L-1	Murine Preadipocyte cell line	MTT assay
39.	Sarsapari la	Smilax Ornata	Rhizome	Reduce joint pain, skin itching, reduce inflammation	T-470, HT-29	Breast cancer, Colon cancer	MTT assay
40.	Punarnav a	Boerhavia Diffusa	Root	Revitalize liver, rheumatoid arthiritis, antiangiogenic	MCF-7	Breast cancer	MTT assay

CONCLUSION

A few Indian origin medicinal plants with anticancer properties have been included in this review. These Indian plants possess anticancer activity due to their strong antioxidant properties. This study's objective is to provide an overview of the developments in India's research on medicinal plants that have anticancer properties. We have made an effort to investigate the newly found plants that have anticancer properties using invitro techniques. India is among the most promising regions in the world for finding new compounds in its flora that are physiologically active. To protect the humans from cancer, more research is required to identify powerful anticancer plants found on Mother Earth.

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