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### *In vitro* and *in vivo* Methods for Anticancer Activity Evaluation and Some Indian Medicinal Plants Possessing Anticancer Properties: An Overview

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Cancer is a major public health burden in both developed and developing countries. Anticancer activity is the effect of natural and synthetic or biological and chemical agents to reverse, suppress or prevent carcinogenic progression. Several synthetic agents are used to cure the disease but they have their toxicity and hence the research is going on to investigate the plant derived chemotherapeutic agents. Therefore an attempt has been made to review different *in vitro* and *in vivo* methods for estimating anticancer properties of natural products from medicinal plants. In this review, 50 anticancer medicinal plants of Indian origin belonging to 35 families are reported along with detailed information regarding part used, extract used, type of the model used, types of tested cancer cell lines, etc. These plants continue to be used against various types of tumours such as sarcoma, lymphoma, carcinoma and leukemia. All these plants are potential candidates for *in vivo* studies since they are showing good *in vitro* anticancer activity.

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**Keyword:** Anticancer Medicinal Plants, Indian origin, Tumours, *in vitro* and *in vivo* Methods.

#### 1. Introduction

Ayurveda, a traditional Indian medical practice using plant drugs has been successful from very early times in using these natural drugs and preventing or suppressing various tumours with different lines of treatment<sup>[1]</sup>. In India, people of different ethnic groups inhabiting various terrains, possess their own distinct culture, religious rites, food habit and a rich knowledge of traditional medicine<sup>[2]</sup>. They practice herbal medicine to cure a variety of diseases. Natural products, especially plants have been used in the treatment of various diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient times and an impressive number of modern drugs have been developed from them. The first written records on the medicinal uses of

plants appeared about 2600 BC from the Sumerians and Akkaidians<sup>[3]</sup>.

Cancer is a group of diseases caused by loss of cell cycle control. Cancer is associated with abnormal uncontrolled cell growth<sup>[4]</sup>. Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). Cancer is a significant worldwide health problem generally due to the lack of widespread and comprehensive early detection methods, the associated poor prognosis of patients diagnosed in later stages of the disease and its increasing incidence on a global scale. Indeed, the struggle to combat cancer is one of the greatest challenges of mankind<sup>[5]</sup>.

The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity<sup>[6]</sup>. Over 3000 species of plants with antitumour properties have been reported<sup>[7]</sup>. Cancer is one of the most prominent diseases in humans and currently there is considerable scientific and commercial interest in the continuing discovery of new anticancer agents from natural product sources<sup>[8]</sup>.

Chemoprevention is recognized as an important approach to control malignancy and recent studies have focused on the search for desirable chemopreventive agents. Natural products, particularly dietary substances, have played an important role in creating new chemopreventive agents<sup>[9]</sup>. Interesting patterns of differential cytotoxicity have been associated with known classes of compounds, such as cardenolides, lignans or quassinoids<sup>[10]</sup>. In any cancer drug discovery program, a paradigm based on ethnobotanical and ethnopharmacological data would be more economical and beneficial in identifying potential anticancer molecules than mass screening of plant species<sup>[11]</sup>. Natural products have been regarded as important sources of potential chemotherapeutic agents and many anticancer drugs have originated from natural sources<sup>[12]</sup>.

According to Cragg and Newman<sup>[13]</sup> over 50 % of the drugs in clinical trials for anticancer properties were isolated from natural sources or are related to them. Several natural products of plant origin have potential value as chemotherapeutic agents. Some of the currently used anticancer agents derived from plants are podophyllotoxin, taxol, vincristine and camptothecin<sup>[14]</sup>. The areas of cancer and infectious diseases have a leading position in utilization of medicinal plants as a source of drug discovery. Among FDA approved anticancer and anti-infectious drugs, drugs from natural origin have a share of 60 % and 75 % respectively<sup>[15]</sup>.

A great number of *in vitro* and *in vivo* methods have been developed to measure the efficiency of natural anticancer compounds either as pure compounds or as plant extracts. *In vitro* methods like, Trypan blue dye exclusion assay, LDH

(Lactic dehydrogenase) assay, MTT assay, XTT assay and Sulforhodamine B assay are most commonly used for estimating anticancer properties of natural products from medicinal plants. Among all *in vitro* methods MTT and Sulforhodamine B assay most popular for estimating anticancer activity.

## 2. Screening methods of anticancer activity:

### 2.1 *In vitro* methods

#### 2.1.1 Trypan blue dye exclusion assay

The trypan blue dye exclusion assay is the most commonly utilized test for cell viability. In this assay, the cells are washed with HBSS (Hank's Buffered Salt Solution) and centrifuged for 10 - 15 min at 10,000 rpm. The procedure is repeated thrice. The cells are suspended in known quantity of HBSS and the cell count is adjusted to  $2 \times 10^6$  cells /ml. The cell suspension is distributed into Eppendorf tubes (0.1 ml containing 2 lakhs cells). The cells are exposed to drug dilutions and incubated at 37 °C for 3 h. After 3 h, dye exclusion test, that is, equal quality of the drug treated cells are mixed with trypan blue (0.4 %) and left for 1 min. It is then loaded in a haemocytometer and viable and non-viable count are recorded within 2 min. Viable cells do not take up colour, whereas dead cells take up colour. However, if kept longer, live cells also generate and take up colour<sup>[16]</sup>. The percentage of growth inhibition is calculated using the following formula:

$$\text{Growth inhibition (\%)} = 100 - \frac{(\text{Total cells} - \text{Dead cells})}{\text{Total cells}} \times 100$$

#### 2.1.2 LDH (Lactic dehydrogenase) Assay<sup>[17]</sup>

Lactic dehydrogenase activity is spectrophotometrically measured in the culture medium and in the cellular lysates at 340 nm by analyzing NADH reduction during the pyruvate-lactate transformation. Cells are lysed with 50 mM 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 g for 15 min. The assay mixture (1ml final volume) for the enzymatic analysis consists of 33 µl of sample in 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.

### 2.1.3 MTT assay<sup>[18]</sup>

The MTT assay, based on the conversion of the yellow tetrazolium salt-MTT, to purple-formazan crystals by metabolically active cells, provides a quantitative determination of viable cells. Cells are plated on to 96 well plates at a cell density of  $2 \times 10^5 \text{ mL}^{-1}$  per well in 100  $\mu\text{L}$  of RPMI 1640 and allowed to grow in  $\text{CO}_2$  incubator for 24 h (37  $^\circ\text{C}$ , 5 %  $\text{CO}_2$ ). The medium is then removed and replaced by fresh medium containing different concentrations of sample for 48 h. The cells are incubated for 24-48 h (37  $^\circ\text{C}$ , 5 %  $\text{CO}_2$ ). Then, 20  $\mu\text{L}$  MTT ([3- (4, 5-dimethylthiazol-yl)-2, 5-diphenyltetrazolium bromide]) stock solution (5 mg/mL in PBS) is added to each well and incubated for 5 h. The medium is removed and 200  $\mu\text{L}$  DMSO is added to each well to dissolve the MTT metabolic product. Then the plate is shaken at 150 rpm for 5 min and the optical density is measured at 560nm. Untreated cells (basal) are used as a control of viability (100 %) and the results are expressed as % viability (log) relative to the control.

### 2.1.4 XTT assay<sup>[19]</sup>

In order to measure the proliferation response, the (2,3-bis[2-Methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt (XTT) assay is used. The tetrazolium salt, XTT, is especially useful in quantifying viable cells. This assay is designed for the spectrophotometric quantification of cell growth and viability without the use of radioactive isotopes and is based on the cleavage of yellow tetrazolium salt, XTT, to form an orange formazan dye by metabolically active cells. XTT cleavages into an orange formazan dye by the mitochondrial enzyme, dehydrogenase, occurs exclusively in living cells. Cells are grown in growth medium plus 10 % FBS in 96-well plates until 70-80 % confluence. They are then treated with the appropriate drug sample for 24 h. An XTT assay is performed at the end of incubation. Briefly, 50  $\mu\text{L}$  of XTT

labeling mixture solution is add to each well, and the cells are incubated at 37  $^\circ\text{C}$  for 4 h. The formazan dye formed is soluble in aqueous solutions and the optical density at 450 nm is compared with that of control wells with a screening multiwell spectrophotometer enzyme-linked immunosorbent assay (ELISA) reader. The reference wavelength is 650 nm.

### 2.1.5 Sulforhodamine B 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 wells overnight, then the samples are added to triplicate wells in serial 3-fold dilutions. Water is added to the control wells at a 1:10 dilution in medium. These plates are incubated at 37  $^\circ\text{C}$ , 5 %  $\text{CO}_2$  for 3 days, then assayed for growth inhibition using a sulforhodamine B (SRB) assay<sup>[20]</sup>. The cells are fixed by the addition of cold 50 % trichloroacetic acid to a final concentration of 10 %. After 1 h incubation at 4  $^\circ\text{C}$ , the cells are washed five times with deionized water. The cells are then stained with 0.4 % SRB (Sigma) 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.

$$\text{The percent growth inhibition is calculated as} = \frac{\text{Control-Sample}}{\text{Control}} \times 100$$

## 2.2 In vivo model

### 2.2.1 Induction of Ehrlich ascites carcinoma<sup>[21]</sup>

Antitumor activity of the test compounds is determined using Ehrlich ascites carcinoma (EAC) tumor model in mice. The ascitic carcinoma bearing mice (donor) are used for the study, 15 days after tumor transplantation. The animals are divided into groups of 12 animals each. ((a) Normal mice (b) Tumor-bearing mice,

(c) Tumor-bearing mice treated with standard drug, (d) Tumor-bearing mice groups treated with test drug) The ascitic fluid is drawn using an 18-gauge needle with sterile syringe. A small amount is testing for microbial contamination. Tumor viability is determine by Tryphan blue exclusion test and cells are counted using haemocytometer. The ascitic fluid is suitably diluted in normal saline to get a concentration of  $10^6$  cells/ml of tumor cell suspension. This is injected intraperitoneally to obtain ascitic tumor. The mice are weighed on the day of tumor inoculation and then once in three days thereafter. Treatment is started on the tenth day of tumor inoculation. Standard (one dose) is injected on tenth day intraperitoneally. The drug is administered from

tenth day for 5 days intraperitoneally. After the administration of last dose followed by 18 h fasting, six mice from each group are sacrifice for the study of antitumor activity and hematological parameters. The remaining animals in each of the groups are kept to check the mean survival time (MST) of the tumor-bearing hosts. Antitumor effects of drug are assessed by observation of following parameters.

- i. Percentage increase in weight as compared to day-0 weight
- ii. Median survival time and increase in lifespan [% ILS]
- iii. Hematological parameters

**Table 1:** List of Indian medicinal plants, their family, part used, solvents used for extraction and assay employed for anticancer studies.

No.	Scientific name (vernacular name, family)	Part/s used	Extract	Type of the tested cancer cells and Method	Traditional and reported uses	Ref. No.
1	<i>Abrus precatorius</i> L. (Chanathi, Fabaceae)	S	50 % ET	Dalton's lymphoma ascites (DLA) cells, small cell lung carcinoma, Yoshida ascites sarcoma, Yoshida sarcoma, mouse fibro sarcoma / <i>In vivo</i> and <i>In vitro</i> / MTT, SRB test	Eye disease, jaundice, poisoning, fainting, arthritis and leucoderma	25,26
2	<i>Allium sativum</i> L. (Lasan, Liliaceae)	P		Oral cancer cell, sarcoma 180 cancer cell / <i>In vivo</i>	Antioxidant properties, anti-asthmatic, anticholesterol-emic, antiseptic, antithrombotic, cancer, cholagogue, diaphoretic and diuretic	38,39
3	<i>Alstonia scholaris</i> L. (Saptaparna, Apocynaceae)	S	85 % EAL	HeLa, hepatocellular carcinoma, promyelocytic leukemia cells, epidermoid carcinoma cell line and breast adenocarcinoma cancer cell lines, Ehrlich ascites carcinoma / <i>In vivo</i> and <i>In vitro</i> / Pratt and Willis test	Antioxidant, diarrhoea, dysentery and treat malaria	27,28
4	<i>Andrographis paniculata</i> Burn.f. (Kariyatu, Acanthaceae)	AP	95 % ET	Lymphocytic, prostate, hepatoma, colon cancer cell lines/ <i>In vitro</i> / MTT test	Antifertility, antihepatic, hepatoprotective, anti-thrombotic, immunostimulant, antihepatotoxic, antiplatelet, aggregation, antihyper-glycaemic, antioxidant, anti-inflammatory and antimalarial	69
5	<i>Annona reticulata</i> L. (Ramfal, Annonaceae)	L	ME	Hepatocellular carcinoma, kidney	Antioxidant, antidysentric, and	70,71

				carcinoma, colorectal carcinoma cancer cell lines / <i>In vitro</i> / MTT test	antihelminthic	
6	<i>Asparagus racemosus</i> Willd. (Shatavari, Liliaceae)	R	AQ	Liver cancer / <i>In vivo</i>	Gastric ulcers, dyspepsia, inflammation, liver diseases and antioxidant	40,41
7	<i>Azadirachta indica</i> Juss. (Neem, Meliaceae)	L	80 % ET	Prostate cancer / <i>In vivo</i>	Immuno modulatory, anti- inflammatory, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties	57,58
8	<i>Bacopa monniera</i> L. (Brahmi, Scrophulariaceae)	WP	9 % ET	Mouse sarcoma Cell line/ <i>In vitro</i> / Trypan blue exclusion test	Mental disorders, tumors, ascites, antioxidant and inflammation	72
9	<i>Bauhinia variegata</i> L. (Kanchhanar, Caesalpiniaceae)	S	95 % ET	Liver cancer cell, epithelial larynx cancer, human breast cancer / <i>In vivo</i> and <i>In vitro</i> line/MTT test	Bronchitis, leprosy, tumors ulcer, antibacterial, antifungal and antioxidant	45
10	<i>Berberis vulgaris</i> L. (Barberry, Berberidaceae)	RB	ME	Breast cancer / <i>In vitro</i> / SRB test	Antioxidant, diarrhea, gallbladder, liver dysfunctions, leishmaniasis, malaria, stomach problems and urinary tract diseases	73
11	<i>Beta vulgaris</i> L. (Beet, Chenopodiaceae)	J	95 % ET	Skin and lung cancer / <i>In vivo</i>	Antioxidant, leukaemia, cancer such as breast, oesophagus, glands, head, intestines and leg	59,60
12	<i>Bidens pilosa</i> L. (Shemaro, Asteraceae)	WP	ME	Cervix carcinoma, nasopharyngeal epidermal carcinoma cancer cell lines / <i>In vitro</i> / MTT test	Antioxidant, wounds, colds, flu and acute or chronic hepatitis urinary tract infections	74,75
13	<i>Calycopteris floribunda</i> Lam. (Bukshi, Kokaranj Combretaceae)	L	DCM:ME (1:1)	Colon cancer cell line / <i>In vitro</i> / MTT test	Colic, antihelminthic, astringent laxative, diarrhoea and malaria	89
14	<i>Catharanthus roseus</i> L. (Sadabahar, barmachi Apocynaceae)	R,L	EA	Acute lymphocytic leukemia / <i>In vivo</i> , Colorectal Carcinoma cell line / <i>In vitro</i> / MTT test	Anti cancer, menorrhagia and antioxidant	42-44
15	<i>Cedrus deodara</i> G. Don (Devdaar, Pinaceae)	W	-	Acute lymphoblastic leukemia, promyelocytic leukemia, prostrate and lung cancer cell lines / <i>In vitro</i> / Trypan blue exclusion test	Astringent, antioxidant, anti diarrhoeal febrifuge, and antiseptic.	90,91
16	<i>Citrullus colocynthis</i> L. (Indrayan, Cucurbitaceae)	L	Glucosides	Breast cancer cell line / <i>In vitro</i> / MTT test	Cytotoxic, hepatoprotective, anti-inflammatory, cardiovascular, antioxidant and anti-diabetic effects	76,77
17	<i>Crocus sativus</i> L. (Kesar, Iridaceae)	dry stigmas	75 % ET	Cervical epithelioid carcinoma cancer cell line / <i>In vitro</i> / MTT test	Antioxidant properties	78,79
18	<i>Curculigo orchoides</i> Gaertn. (Kalimusli, Amaryllidaceae)	R	HE, CH, AN and ME	Breast cancer cell line / <i>In vitro</i> / MTT test	Antioxidant, diarrhoea, jaundice, asthma and poultice for itch and skin diseases	80,81
19	<i>Curcuma longa</i> L. (Haldi, Zingiberaceae)	Rh	-	Colon Cancer Cells / <i>In vitro</i> / Lactate	Antimutagenic, anticarcinogenic,	92,93

				dehydrogenase test	antigenotoxic, anti-inflammatory and antioxidant properties	
20	<i>Cymbopogon flexuosus</i> (Steud.) Wats. (Lemon grass, Poaceae)	G		Colon, cervix, oral, prostate, promyelocytic and leukemia cancer cell lines / <i>In vitro</i> and <i>In vivo</i> / SRB test	Stress-related disorders, antifungal and antimicrobial properties	29
21	<i>Emblica officinalis</i> Gaertn. (Amla, Euphorbiaceae)	DFr	ME	Liver cancer/ <i>In vivo</i>	Liver protecting activity, antimutagenic, antioxidant and anticarcinogenic properties	61,62
22	<i>Ephedra sinica</i> Stapf (Ephedra, Ephedraceae)	AP	ME	Murine melanoma cancer / <i>In vivo</i>	Colds, fever, flu, headaches, asthma, wheezing, and nasal congestion	63
23	<i>Indigofera aspalathoides</i> (Vahl, Papilionaceae)	S	95% ET	Ehrlich's ascites carcinoma cancer / <i>In vivo</i>	Antioxidant, various skin disorders and cancer	45,46
24	<i>Ipomoea aquatica</i> Forskal. (Kalmisag, Convolvulaceae)	L	ME	Larynx epithelial carcinoma, small lung carcinoma cancer and normal African green monkey kidney cell line / <i>In vitro</i> / MTT and SRB test	Antioxidant properties	82
25	<i>Ipomoea squamosa</i> (Cairo Morning Glory, Convolvulaceae)	L	-	Ovarian cancer cell line / <i>In vitro</i>	-	94
26	<i>Jatropha curcas</i> L. (Ratanjota, Huphorbiaceae)	S	ME	Skin cancer / <i>In vivo</i>	Skin diseases, antioxidant, ulcers, tumours	67,68
27	<i>Lantana camara</i> L. (Ghaneri, Verbenaceae)	F,Fr.,L,R, S	ME	Lung carcinoma cell line / <i>In vitro</i> / MTT and SRB test	Antitumoral, antioxidant, antibacterial and antihypertensive	83
28	<i>Mangifera indica</i> L. (Keri, Anacardiaceae)	Fr, B,L		Lung cancer / <i>In vivo</i>	Antitumour, antioxidant, antiviral, antibacterial, analgesic, anti-inflammatory, antidiarrhoeal, antiameobic, spasmolytic, immunostimulant and immunomodulatory properties	47
29	<i>Melia azedarach</i> L. (White Cedar, Meliaceae)	L	70 % ET	Lung cancer and glioma cancer cell line / <i>In vitro</i> / standard Cell Counting Kit (CCK)-8 test	Antiparasitic activity, anthelmintic activity	95,96
30	<i>Morinda citrifolia</i> L. (Noni, Rubiaceae)	R, Fr.	AQ	Colon cancer cell line / <i>In vitro</i> / MTT test	Antidiabetic, antiviral, antibacterial, anticancer and antioxidant	97,98
31	<i>Moringa oleifera</i> L. (Saragavo, Moringaceae)	S	ME, ET, EA and CH	Skin cancer/ <i>In vivo</i> and <i>In vitro</i> / Natural red dye test	Antioxidant, antimicrobial, antigenotoxic and anti-inflammatory activities	36,37
32	<i>Nigella sativa</i> L. (Black seeds, Ranunculaceae)	S	90 % ET	Colon Cancer / <i>In vivo</i>	Antioxidant, antidiabetic, antihistaminic, antiepileptogenic, antiinfective, antitumour and antiperoxidative	48,49
33	<i>Ocimum gratissimum</i> L. (Damro, Lamiaceae)	S, L	AQ	Breast cancer / <i>In vivo</i> and <i>In vitro</i> / MTT test	Chemopreventive, anticarcinogenic, radioprotective and numerous others pharmacological uses	30

34	<i>Ocimum sanctum</i> L. (Tulsi, Lamiaceae)	L	ET	Skin cancer / <i>In vivo</i>	Anti-stress, antioxidant, hepatoprotective, anti-inflammatory, antibacterial and radioprotective properties	64
35	<i>Phellinus rimosus</i> (Berk, (Hymenochetaceae)	sporocarps	ME, AQE	Dalton's lymphoma ascites, Ehrlich's ascites carcinoma / <i>In vivo</i> and <i>In vitro</i> / Trypan blue exclusion test	Antioxidant	31,32
36	<i>Pinus resinosa</i> Aiton (Pinaceae)	W	HE, DCM, ME and AQ	Colorectal adenocarcinoma cell, lung carcinoma cell and normal skin Fibroblast cell lines / <i>In vitro</i> / Resazurin reduction test	Antioxidant, analgesic, antifungal and antibacterial	84,85
37	<i>Polyalthia longifolia</i> Benth. & Hook. f. (Annonaceae)	L	ET	Colon cell and leukemia HL-60 cancer cell line / <i>In vitro</i> / SRB test	Antibacterial and antifungal activities	99
38	<i>Psidium guajava</i> L. (Jamphal, Myrtaceae)	L	AQ	Prostate carcinoma cell / <i>In vitro</i> / MTT test	Antioxidant	100
39	<i>Punica granatum</i> L. (Dadam, Lythraceae)	J,P	70 % AC	Prostate carcinoma cell / <i>In vivo</i> and <i>In vitro</i> / MTT test	Antioxidant and anti-inflammatory	33
40	<i>Tragia involucrata</i> Linn. (Euphorbiaceae)	AP	HE, EA	Ehrlich's ascites carcinoma/ <i>in vivo</i>	Antimicrobial, antiinflammatory, antifertility activity	101
41	<i>Rubia cordifolia</i> L. (Manjistha, Rubiaceae)	R	80 % ME	Coloncancer, breast carcinoma and liver carcinoma / <i>In vitro</i> / MTT test	Antitumor, antioxidant, anti inflammatory, urinary disorders, antistress, anti microbial, hepatoprotective, radio protective	86,87
42	<i>Semecarpus anacardium</i> L. (Bhallika, Anacardiaceae)	DFr	90 % ET and ME	Acute myeloblastic leukaemia, chronic myelogenic leukaemia, breast adenocarcinoma, cervical epithelial carcinoma and colon carcinoma cancer cell lines / <i>In vitro</i> / MTT test	Antioxidant, immunomodulatory, antiinflammatory, analgesic, antipyretic and ulcerogenic activities	88,11
43	<i>Tephrosia purpurea</i> Pers. (Sarapunkha, Fabaceae)	R	95 % ET	Oral squamous cell carcinoma / <i>In vivo</i>	Various inflammatory, liver, spleen and kidney disorders and antioxidant	50
44	<i>Terminalia chebula</i> Retz. (Karakkaya, Combretaceae)	F	ET	COLO-205 cell line / <i>In vitro</i> / MTT test	Digestive, diabetes, colic pain, chronic cough, sore throat, asthma, antioxidant, anti-inflammatory	102
45	<i>Tiliacora racemosa</i> Coleb. (Tiliacoru, Menispermaceae)	R	90 % ET	Acute myeloblastic leukaemia, chronic myelogenic leukaemia , breast adenocarcinoma and cervical epithelial cancer cell lines / <i>In vitro</i> / MTT test	-	88
46	<i>Tinospora cordifolia</i> (Willd.) Hook. f. & Thom. (Guduchi, Menispermaceae)	S	PE, CH and DCM	Ehrlich's ascites carcinoma / <i>In vivo</i>	General tonic, antioxidant, anti-inflammatory, antiarthritic, antiallergic, anti-malarial, anti-diabetic and aphrodisiac properties	51,52
47	<i>Viscum album</i> L. (Vando,	L	CO <sub>2</sub> gas	Ehrlich's tumour cell / <i>In vivo</i>	Nervine, hypotensive, cardiac depressant, antioxidant, vasodilator,	66

	Viscaceae)				relaxant, diuretic and stimulant	
48	<i>Withania somnifera</i> L. (Ashwagandha, Solanaceae)	R	70 % EAL	Forestomach and skin carcinoma cancer / <i>In vivo</i>	Antitumor, radiosensitizer, antioxidant, antistressor, immunomodulatory, anti-inflammatory and anti-bacterial	53,54
49	<i>Woodfordia fruticosa</i> Salisb. (Dhavdi, Lythraceae)	F	70 % AC	Sarcoma 180 cancer / <i>In vivo</i>	Antipyretic, antioxidant, Anti-inflammatory, hepato-protective, antibacterial activity	55,56
50	<i>Zingiber officinale</i> Rosc. (Adu, Zingiberaceae)	Rh	50 % ET	Prostate cancer cellline / <i>In vitro</i> and <i>In vivo</i> / MTT test	Carminative, antioxidant, diaphoretic, antispasmodic, expectorant, peripheral circulatory stimulant, astringent, appetite stimulant, anti-inflammatory agent, diuretic and digestive	34,35

S: Stem, P: Peel, AP: Aerial parts, L: leaves, R: root, WP: whole plant, RB: rootbark, J: juice, W: wood, Rh: rhizomes, G: grass, DFr: dry fruits, F: flower, B: bark

ET: Ethanol, EAL: Ethyl alcohol, ME: Methanol, AQ: Aqueous, DCM: dichloromethane, EA: Ethyl acetate, HE: Hexane, CH: Chloroform, AN: Acetonitrile, AC: Acetone.

### 2.2.2 Anticancer Medicinal Plants of India

Anticancer properties of many natural compounds isolated from different Indian plant extracts have been reported. Research is being carried out throughout the world to find a lead compound which can block the development of cancer in humans. Nature has always been a great contributor towards this goal. Plant-derived natural products such as flavonoids, terpenoids and steroids have received considerable attention due to their diverse pharmacological properties, which include cytotoxic and chemopreventive effects<sup>[22]</sup>. The isolation of the vinca alkaloids, vinblastine and vincristine from the Madagascar periwinkle, *Catharanthus roseus* introduced a new era in the use of plant material as anticancer agents. They were the first agents to advance into clinical use for the treatment of cancer<sup>[23]</sup>.

The medicinal plants contain many antioxidants such as vitamins (A, C, E, K), carotenoids, flavonoids (flavones, isoflavones, flavonones, anthocyanins, catenches, isocatechins), polyphenols (ellagic acid, gallic acid, tannins), saponins, enzymes and minerals (selenium, copper, manganese, zinc, chromium, iodine, etc)<sup>[24]</sup>.

In this review, 50 anticancer medicinal plants of Indian origin belonging to 35 families are reported along with detailed information

regarding part used, extract used, type of the model used, types of tested cancer cell lines, etc. (Table-1). These plants continue to be used against various types of tumours such as sarcoma, lymphoma, carcinoma and leukemia. Many of these medicinal plants have been found to be very effective in experimental as well as clinical cases of tumours/cancers.

Some medicinal plants have been studied in various *in vivo* and *in vitro* experimental models of cancer and have shown significant inhibition of cancer cell proliferation. For eg. *Abrus precatorius* in Yoshida's sarcoma, carcinoma and Dalton's lymphoma ascites cancer<sup>[25,26]</sup>; *Alstonia scholaris* in Ehrlich ascites carcinoma<sup>[27,28]</sup>; *Cymbopogon flexuosus* in Ehrlich ascites carcinoma, leukemia and sarcoma 180<sup>[29]</sup>; *Ocimum gratissimum* in breast cancer<sup>[30]</sup>; *Phellinus rimosus* in lymphoma and carcinoma<sup>[31,32]</sup>; *Punica granatum* in prostate cancer<sup>[33]</sup>; *Zingiber officinale* in carcinoma<sup>[34, 35]</sup>; *Moringa oleifera* in skin cancer and Human multiple myeloma cancer<sup>[36,37]</sup>; *Allium sativum* in sarcoma 180<sup>[38,39]</sup>; *Asparagus racemosus* in liver cancer<sup>[40,41]</sup>; *Catharanthus roseus* in P-1534 leukemia<sup>[42-44]</sup>; *Indigofera aspalathoides* in Ehrlich's ascites carcinoma<sup>[45,46]</sup>; *Mangifera indica* in lung cancer<sup>[47]</sup>; *Nigella sativa* in colon cancer<sup>[48,49]</sup>; *Tephrosia purpurea* in oral

carcinoma<sup>[50]</sup>; *Tinospora cordifolia* in Ehrlich's ascites carcinoma<sup>[51,52]</sup>; *Withania somnifera* in skin carcinoma<sup>[53,54]</sup>; *Woodfordia fruticosa* in sarcoma 180<sup>[55,56]</sup>; *Azadirachta indica* in prostate cancer<sup>[57,58]</sup>; *Beta vulgaris* in skin and lung cancer<sup>[59,60]</sup>; *Embllica officinalis* in liver cancer<sup>[61,62]</sup>; *Ephedra sinica* in Murine melanoma<sup>[63]</sup>; *Ocimum sanctum* in skin cancer<sup>[64]</sup>; *Viscum album* in Ehrlich's carcinoma<sup>[65,66]</sup>; *Jatropha curcas* in skin cancer<sup>[67,68]</sup>; *Andrographis paniculata* in lymphoma and carcinoma<sup>[69]</sup>; *Annona reticulate* in kidney and colorectal carcinoma cancer<sup>[70,71]</sup>; *Bacopa monniera* in sarcoma<sup>[72]</sup>; *Berberis vulgaris* in breast cancer<sup>[73]</sup>; *Bidens pilosa* in cervix cancer<sup>[74,75]</sup>; *Citrullus colocynthis* in breast cancer<sup>[76,77]</sup>; *Crocus sativus* in cervical epithelioid carcinoma cancer<sup>[78,79]</sup>; *Curculigo orchioides* in breast cancer<sup>[80,81]</sup>; *Ipomoea aquatica* in larynx epithelial carcinoma and small lung carcinoma cancer<sup>[82]</sup>; *Lantana camara* in lung carcinoma<sup>[83]</sup>; *Pinus resinosa* in Colorectal adenocarcinoma cell, lung carcinoma cell and normal skin Fibroblast<sup>[84,85]</sup>; *Rubia cordifolia* in carcinoma<sup>[86,87]</sup>; *Tiliacora racemosa* in leukaemia and carcinoma<sup>[88]</sup>; *Calycopteris floribunda* in colon cancer<sup>[89]</sup>; *Cedrus deodara* in acute lymphoblastic leukemia, prostate and lung cancer<sup>[90,91]</sup>; *Curcuma longa* in colon cancer<sup>[92,93]</sup>; *Ipomoea squamosa* in ovarian cancer<sup>[94]</sup>; *Melia azedarach* in lung cancer and glioma cancer<sup>[95,96]</sup>; *Morinda citrifolia* in colon cancer<sup>[97,98]</sup>; *Polyalthia longifolia* in colon and leukemia HL-60 cancer<sup>[99]</sup>; *Psidium gujava* in prostate carcinoma cancer<sup>[100]</sup>; *Tragia involucrata* in carcinoma cancer<sup>[101]</sup>; *Semecarpus anacardium* acute myeloblastic leukaemia, chronic myelogenic leukaemia, breast adenocarcinoma, cervical epithelial carcinoma and colon carcinoma cancer<sup>[88,111]</sup>; *Terminalia chebula* in colon cancer<sup>[102]</sup>.

This review provides information on a number of plants which show promising anticancer activity. It lists various methods for evaluating anticancer activity so it will be easy for the experimenter. It emphasizes that *in vitro* anticancer assays have been carried out for most of the plants, but *in vivo* remains to be done in majority of them.

### 3. Conclusion

In this review, some anticancer medicinal plants of Indian origin have been presented. These medicinal plants possess good antioxidant properties, leading to anticancer activities. The aim of this study was to give an overview on the progress of anticancer medicinal plant research around the continental India, focusing on the most important findings of scientists in this field. We have tried to explore the discovered plants components with proved anticancer activity both *in vitro* and *in vivo*. India is one of the most promising regions for discovering novel biologically-active substances from its flora. More efforts are needed to explore potent anticancer plants from the mother earth and save humans around the world from cancer.

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