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ANTICANCER POTENTIAL OF MEDICINAL PLANTS *WITHANIA SOMNIFERA*, *TINOSPORA CORDIFOLIA* AND *CURCUMA LONGA*: A REVIEW

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Abstract- Cancer is a hyperproliferative disorder that involves transformation, dysregulation of apoptosis, proliferation, invasion, angiogenesis and metastasis. Because of high death rate associated with cancer and because of serious side effects of chemotherapy and radiation therapy, many cancer patients seek complementary and alternative methods of treatment. Although more than 1500 anticancer drugs are in active development with over 500 drugs under clinical trials, there is still a need to develop more effective and less toxic drugs through the heritage of Ayurveda. Plants have been used for treating diseases since time immemorial. The recent research findings give valuable information that herbs such as *Withania somnifera* (Ashwagandha), *Tinospora cordifolia* (Guduchi) and *Curcuma longa* (Haldi) possess anti-carcinogenic properties and are useful at various levels. Review of literature was done by searching books and articles published in English on the above medicinal plants and indexed in MEDLINE, EMBASE and EBSCO medical databases. In the present review, an attempt has been made to review recent research findings on the role of the above medicinal plants as anticancer agents for the benefit of mankind as well as draw attention of young, brilliant minds who want to explore the field of Naturopathy and Ayurveda.

Keywords- Anticancer, Cytotoxicity, *Curcuma longa, Tinospora cordifolia*, *Withania somnifera*

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Introduction

The incidence of cancer has been rising alarmingly for the last few decades. In India, cancers of oral cavity, oro-pharynx, oesophagus, stomach, rectum, colon and lung are commonly seen in men, whereas cancers of the cervix and breast commonly affect Indian women. In spite of technical advancements in the diagnosis and management, cancer still remains a major health care burden throughout the globe. Therefore, there is a burning need for a safe and effective alternative, which relies heavily on medicinal plants. Ayurveda is a storehouse of knowledge in the field of immunology and many medicinal plants have been prescribed for this condition.

Ayurveda, which means the 'Science of Life' is the oldest medical science in the Indian subcontinent and has been practiced since the 12th century BC. Ayurveda is not merely a system of medicine: rather, it is a way of life. Its objective is to accomplish physical, mental, social and spiritual well-being by adopting preventive and promotive approaches as well as treating diseases with a holistic approach.

Charaka [1] and *Sushruta* [2] *samhitas*, two well-known Ayurvedic classics, describe cancer as inflammatory or non-inflammatory swelling and mention them as either '*Granthi*' (minor neoplasm) or '*Arbuda*' (major neoplasm).The therapeutic approach of Ayurveda has been divided into four categories as *Prakritisthapani chikitsa* (health maintenance), *Roganashani chikitsa* (disease cure), *Rasayana chikitsa* (restoration of normal function) and *Naishthiki chikitsa* (spiritual approach) [3]. During the 7th century BC, Atreya and Dhanwantari used herbal medicines for treating the early stages of cancer and surgery in advanced cases. In the 8th century AD, Vagbhata, a Buddhist physician composed two texts: *Astanga Hrdaya* [4] and *Astanga sangraha* [5] where new methods for cancer treatment were introduced. Other Ayurvedic texts of internal medicine, *viz.*, *Chakradatta* [6] composed by Chakrapani (10th century AD), the *Sarangadhara Samhita* [7] by Sarangadhara (14th century AD), the *Bhavaprakasha Samhita* [8] by Bhavamisra (15th century AD), the *Satmya Darpan Samhita* by Viswanath (16th century AD), the *Vaisajya Ratnabali* by Binoda Lala Sen Gupta (18th century AD), the *Rasatarangini* by Sadananda Sharma (19th century AD), etc., explain numerous remedies to treat internal and external neoplasms.

Many such recommendations of Ayurvedic texts have been scientifically validated. Medicinal plants *Withania somnifera*, *Tinospora cordifolia* and *Curcuma longa* are routinely used in traditional methods employed in treatment of various cancers [9, 10]. In addition to these traditional methods, various herbal combinations of these plants have been mentioned in Ayurvedic texts [11-13]. The present review presents the scientific and clinical studies conducted to evaluate the efficacy of these important medicinal plants *viz. Withania somnifera*, *Tinospora cordifolia* and *Curcuma longa* in prevention and treatment of various kinds of cancer.

Methods

The literature review was limited to books, articles published in English, and indexed on MEDLINE, EMBASE and EBSCO medical databases. Keywords used in the search included: *Withania somnifera*, ashwagandha, *Tinospora cordifolia*, giloy, guduchi, *Curcuma longa,* haldi, anticancer, cytotoxicity. The results of the database search were reviewed to identify relevant articles.

Withania somnifera (WS)

Ashwagandha [Fig-1] is commonly known as "Indian Winter cherry" or "Indian Ginseng". The root smells like horse ("ashwa") because of which it is called ashwagandha. The species name *somnifera* means "sleep-inducing" in Latin, indicating its sedative properties.

Classification of *Withania somnifera*

Kingdom: Plantae
Division: Angiosperms
Class: Dicotyledonae

Order: Solanales
Family: Solanaceae
Genus: *Withania*
Species: *somnifera*

(a) (b)

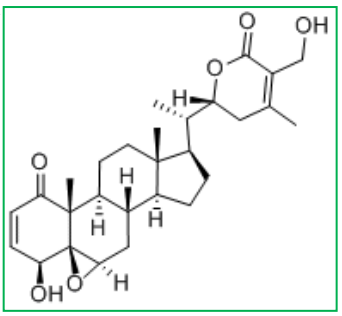
(c) (d)

(e)

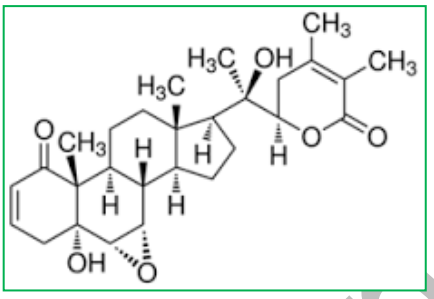
Fig-1 (a) *Withania somnifera* (L.) Dunal Habit (b) Leaves (c) Flower (d) Fruit (e) Stem.

Image courtesy naturesalive.wordpress.com, www.nmpb.nic.in, www.flowersofindia.net and Jain et al., 2012 [14]

Active ingredients: Alkaloids (isopelletierine, anaferine, cuscohygrine, anahygrine, etc.), steroidal lactones (withanolides, withaferins) and saponins [15]. Withaferin A (WFA) and Withanolide A [Fig-2] derived from this medicinal plant, have been reported for its anti-tumorigenic activity against various cancer cells [16].



(a)



(b)

Fig-2 Structures of important withanolides: (Withaferin A and (b) Withanolide A.

Image courtesy www.chemicalbook.com and www.sigmaaldrich.com.

Anti- cancer activity of WS

Several studies have been conducted to evaluate the effectiveness of WS in prevention and treatment of various cancers, which are detailed below:

a. Colon Cancer

***In vitro* studies:**

Koduru et al., (2010) have observed the anticancer activity of WFA, which exhibits potential for further development for targeted chemotherapy and/or chemoprevention strategies in the context of colon cancer [17]. Studies have been carried out to isolate twelve withanolides from the leaves of this plant [Table-1]. Thus, it is hypothesized that incorporation of withanolides in the diet may prevent or decrease the growth of tumors in humans [18].

Table-1 *Cytotoxic Activity of Withanolides from leaves of WS against HCT 16 (human colorectal carcinoma cell line). Adapted from Koduru et al., 2010 [17].*

S. No.	Withanolides	IC_{50} values (µg/ml)
1.	Withaferin A	0.24 ± 0.01- 11.6 ± 1.9
2.	Sitoindoside IX	
3.	4-(1-hydroxy-2, 2-dimethylcyclpropanone)-2,3-dihydrowithaferin A	
4.	2, 3-dihydrowithaferin A	
5.	24, 25-dihydro-27-desoxywithaferin A	
6.	physagulin D (1-->6)-β-Dglucopyranosyl-(1-->4)-beta-D-glucopyranoside	
7.	27-O-β-D-glucopyranosylphysagulin D	
8.	physagulin D	
9.	withanoside IV	
10.	27-O-β-D-glucopyranosylviscosalactone B	7.9 ± 2.9-17.3 ± 3.9
11.	4,16-dihydroxy-5beta,6-β-epoxyphysagulin D	
12.	viscosalactone B	0.32 ± 0.05-0.47 ± 0.15

In vitro cytotoxicity of 50% ethanolic extract of root, stem and leaves of WS has been evaluated against HCT-15 (human colorectal adenocarcinoma cell line). Results have shown that root, stem and leaves extracts of WS possess cytotoxic activity ranging from 0-98% depending on the cell lines but maximum activity has been found in 50% ethanolic extract of leaves [19].

***In vivo* studies:**

A study by Muralikrishnan et al (2010) has revealed that azoxymethane induced experimental colon cancer and immune dysfunction in mice is controlled by WS [20]. In another study, Muralikrishnan et al (2010) have observed that WS decreases the activities of key TCA cycle enzymes in colon cancer bearing animals [21].

b. Lung Cancer

***In vitro* studies**

Leaf extracts of WS have been shown to exhibit cytotoxic properties against lung cancer cell lines [18]. Compounds 1-12 and diacetylwithaferin A extracted from leaves

have been tested for their antiproliferative activity on NCI-H460 (human large-cell lung carcinoma cell line). This cytotoxicity is comparable to that achieved with the common cancer chemotherapy drug doxorubicin (Caelyx®, Myocet®). In fact, researchers have reported that WFA is more effective than doxorubicin in inhibiting breast and colon cancer cell growth [18,22]. Studies have shown that root, stem and leaves extracts of WS possess cytotoxic activity against A-549 (human lung carcinoma cell line) [19]. WS has been found to be beneficial in lung cancer.

***In vivo* studies**

WS has been shown to possess antitumor activity against urethane-induced lung-adenomas in adult male albino mice by inducing nonspecific increase in resistance [23]. Pharmacokinetic studies in mice have revealed that WFA reaches peak concentrations of up to 2 µM in plasma with a half-life of 1.36 h following a single 4 mg/kg dose. In a breast cancer metastasis mouse model, WFA has been shown to exhibit a dose-dependent inhibition of metastatic lung nodules with minimal toxicity to lung tissue [24]. In another study, the combination of paclitaxel and WS has been shown to effectively treat the benzo-(a)-pyrene-induced lung cancer in mice by offering protection from ROS damage and also by suppressing cell proliferation [25].

c. Blood Cancer

***In vitro* studies**

Malik et al (2007) have observed that WFA, the major chemical constituent of WS, primarily induces oxidative stress in human leukemia HL-60 cells and in several other cancer cell lines and triggers events responsible for mitochondrial-dependent and -independent apoptosis pathways [26]. Another study has demonstrated that Withanolide D (C4β-C5β,C6β-epoxy-1-oxo-,20β, dihydroxy-20S,22Rwitha-2,24-dienolide; WithaD), a pure herbal compound isolated from WS has capability to induce apoptosis in a dose and time dependant manner both in myeloid K562 (human bone marrow lymphoblast cell line) and lymphoid MOLT-4 (human bone marrow T lymphoblast cell line) cells. Taken together, this pure herbal compound (WithaD) may be considered as a potential alternative tool with additive effects in conjunction with traditional chemotherapeutic treatment, thereby accelerating the process of conventional drug development [27]. Oza et al (2010) have studied anticancer properties of highly purified L-asparaginase from WS against acute lymphoblastic leukemia [28].

d. Skin Cancer

***In vivo* studies**

Scientific studies conducted in mice have revealed that the roots of WS have capability to inhibit fore-stomach and skin carcinogenesis in mice [29]. Mathur et al (2004) have observed that 1-oxo-5-β-6-β-epoxy-witha-2-enolide, a chemical constituent isolated from the root of WS, has the potential of acting as an effective agent to prevent the incidence of skin carcinoma induced by ultra violet radiation [30]. The chemopreventive effect of WS hydroalcoholic root extract (WSRE) on 7,12-dimethylbenz-[a]-anthracene (DMBA) induced skin cancer has been investigated in Swiss albino mice and the results of the study have revealed a significant decrease in incidence and average number of skin lesions in mice compared with DMBA alone at the end of 24th week. Further, a significant impairment has also been noticed in the levels of reduced glutathione, malondialdehyde, superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase (GST) in skin lesions of DMBA-treated control mice compared with vehicle-treated mice. This study has proven that WRSE possesses potential chemopreventive activity and this activity may be linked to the antioxidant/free radical-scavenging constituents of the extract [31]. Davis and Kuttan (2001) have observed that administration of an extract of WS reduces two-stage skin carcinogenesis induced by DMBA (dimethyl benzanthracene) and croton oil. Enzyme analysis of skin and liver has shown significant enhancement in antioxidant enzymes such as GST, glutathione peroxidases and catalases in WS treated group as compared to control. The elevated level of lipid peroxide in the control group is significantly inhibited by WS administration. These studies indicate that WS has the potential to reduce the papilloma-induced alterations by its antioxidant defense systems [32].

e. Breast Cancer

***In vitro* studies**

Compounds 1-12 and diacetylwithaferin A from leaves of WS have also been tested on MCF-7 (human breast tumorigenic, ER$^+$ and non-invasive cell line) [18, 19]. WFA has also been found to inhibit growth of MDA-MB-231 (human breast carcinoma, ER$^-$, tumorigenic and invasive cell line) xenografts *in vivo* by causing apoptosis. WFA, a vimentin cytoskeleton inhibitor, has been found to be a potent breast cancer antimetastatic agent and the anti-metastatic activity of WFA is, at least in part, mediated through its effects on vimentin and vimentin ser^{56} phosphorylation [24].

In addition, these studies indicate that WA functions as an antiestrogen and the pro-apoptotic effect of this promising natural product is partially attenuated by p53 knockdown and E2-ER-α [33]. WS has been found to inhibit constitutive as well as interleukin-6 (IL-6)-inducible activation of signal transducer and activator of transcription 3 (STAT3), which is an oncogenic transcription factor activated in many human malignancies including breast cancer. The IL-6-stimulated activation of STAT3 conferred a modest protection against WA-mediated suppression of MDA-MB-231 cell invasion. The results of the study indicate that WA can trigger apoptosis and largely inhibit cell migration/invasion of breast cancer cells even after IL-6-induced activation of STAT3, which should be viewed as a therapeutic advantage for this agent [34]. A novel bioactive compound called withanolide sulfoxide obtained from methanol extract of WS roots has been shown to suppress human tumor cell proliferation and its IC^{50} value against MCF-7 (human breast cancer cell line) is in the range of 0.74-3.63 µM. In addition, S-containing dimeric withanolides have also been found to completely suppress TNF-induced NF-κB activation when tested at 100 µM [35]. In-vitro cytotoxic effects of aqueous and ethanolic extracts of stem of WS have also been studied on MCF-7 and MDA-MB-231 cells [36].

f. Renal Cancer

***In vitro* studies**

Yang et al have reported WFA enhanced radiation-induced apoptosis in human renal cancer cells (Caki) cells through ROS generation, down-regulation of Bcl-2 and Akt dephosphorylation [37]. In another study, treatment of Caki cells with WFA has been demonstrated to induce a number of signature ER stress markers, including phosphorylation of eukaryotic initiation factor-2α (eIF-2α), ER stress-specific X-box binding protein 1 (XBP1) splicing, and up-regulation of glucose-regulated protein (GRP)-78. In addition, WFA has been shown to cause up-regulation of CAAT/enhancer-binding protein homologous protein (CHOP), thereby suggesting the induction of ER stress. Pretreatment with N-acetyl cysteine (NAC) significantly inhibits WFA-mediated ER stress proteins and cell death, suggesting that reactive oxygen species (ROS) mediate WFA-induced ER stress [38].

Furthermore, it has been shown that CHOP siRNA or inhibition of caspase-4 activity attenuates WFA-induced apoptosis. Taken together, there is strong evidence supporting an important role of the ER stress response in mediating WFA-induced apoptosis [38].

g. Fibrosarcoma

***In vitro* studies**

A study revealed that the extract from WS has an IC^{50} value at 24 h of 150 and 60 µg/ml, on L929sA (murine fibrosarcoma cell line, derived from L929) [39].

***In vivo* studies**

Chemopreventive studies of hydro-alcoholic extract of WS roots, against 20-methylcholanthrene induced fibrosarcoma tumors in Swiss albino mice have revealed that WS extract (one week before injecting 20-methylcholanthrene and continued until 15 weeks thereafter) significantly reduces tumor incidence, tumor volume and enhances the survival of the mice, as compared to 20-methylcholanthrene injected mice. The tumor incidence is also delayed in the treatment group when compared with 20-methylcholanthrene injected mice.

A significant modulation of reduced glutathione, lipid peroxides, GST, catalase and superoxide dismutase in extract treated mice compared with 20-methylcholanthrene injected mice has also been recorded. These studies indicate that chemopreventive activity of WS extract may be due to its antioxidant and detoxifying properties [40]. In another study, administration of an extract from the root of the plant WS (20mg/dose/animal i.p.) has been found to inhibit the 20-methylcholanthrene induced sarcoma development in mice and also to increase the life span of tumor bearing

animals and inhibition of the lipid peroxide formation (152 nM/mg protein) ($P<0.01$) as compared with control (198 nM/mg protein) [41].

h. Prostate cancer

***In vitro* studies**

A study conducted to evaluate *in vitro* cytotoxic of 50% ethanol extract of root, stem and leaves of WS against PC-3 (human metastatic prostate cell line derived from bone) and DU-145 (human metastatic prostate cell line derived from brain) has revealed that root, stem and leaf extracts show cytotoxicity activity on the above two cell lines. Further, ethanol extracts of leaves obtained from various treatments show strong activity against PC-3 with 80-98% growth inhibition [19].

i. Pancreatic Cancer

***In vitro* studies**

A study conducted to investigate the efficacy and mechanism of Hsp90 inhibition by WFA in pancreatic cancer *in vitro* and *in vivo*, has revealed that WFA acts as a potent antiproliferative agent against pancreatic cancer cell lines Panc-1, MiaPaCa2 and BxPc3, respectively [42].

***In vivo* studies**

It has been demonstrated that WFA binds and inhibits Hsp90 chaperone activity through an ATP independent mechanism that results in Hsp90 client protein degradation and thus, exhibits *in vivo* anticancer activity against pancreatic cancer [42].

j. Neuroblastoma

***In vitro* studies**

Root, stem and leaves extracts of WS have been evaluated against IMR-32 (human metastatic neuroblastoma cell line derived from abdominal mass) and SF-268 (CNS anaplastic astrocytomal non epithelial cell line) and were found to possess significant cytotoxic activity against these cell lines [18,19].

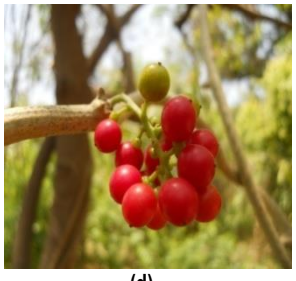
Tinospora cordifolia (TC)

Tinospora cordifolia [Fig-3], also known as Giloy, Guduchi or Amrita, is used in the treatment of various diseases in the traditional medicinal system in India such as Ayurveda, Yoga & Naturopathy Unani, Siddha and Homeopathy (AYUSH) and is also a known immune system modulator. The deciduous climbing shrub has shown a great potential for the development of industrial products and commercial exploitation of biopharmaceuticals for the treatment of various diseases [43]. The focus of the present review is to galvanize the potential of this medicinal shrub as an anticancer agent.

(a)

(b)

(c)



(d)

(e)

Fig-3. (a) *Tinospora cordifolia (Willd.) Hook. F. and Thoms.* Habit (b) Leaves (c) Flower (d) Fruits (e) Stem.

Image courtesy naturesalive.wordpress.com, www.nmpb.nic.in, www.flowersofindia.net and Mittal et al., 2014 [44]

Classification of *Tinospora cordifolia*

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Ranunculales
Family: Menispermaceae
Genus: *Tinospora*
Species: *cordifolia*

Active ingredients: Alkaloids (berberine, tinosporin) [Fig-4], diterpenoid lactones, glycosides, sesquiterpenoid, aliphatic compounds, phenolics, polysaccharides, steroids like tinosporide, tinosporaside, cordifolide, cordifol, heptacosanol, clerodane furano diterpene, diterpenoid furanolactone tinosporidine, columbin and β-sitosterol.

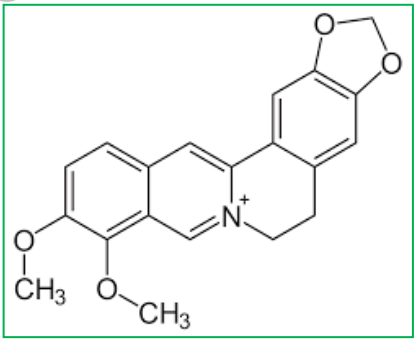


Fig-4 Structure of alkaloid Berberine.

Image courtesy www.en.wikipedia.org.

Anti- cancer activity of TC:

TC has been shown to possess anticancer activity against various tumors/cancers as detailed below:

a. Colon cancer

***In vitro* studies**

Aqueous fraction of stem has shown potent cytotoxic activity against human colon cancer cell lines namely Colo-205 (human colorectal metastatic carcinoma cell line derived from ascites) and HCT-116 (human colorectal carcinoma cell line) [45].

b. Lung Cancer

***In vitro* studies**

Aqueous fraction of TC stem has been found to exhibit potent cytotoxic activity against lung cancer cell lines A-549 and NCI-H322 (human bronchio-alveolar metastatic carcinoma cell line derived from cervical node) [45].

***In vivo* studies**

There has been found to be a 72% inhibition in the metastases formation in the lungs of syngeneic C57BL/6 mice, when TC was administered simultaneously with tumor challenge. Biochemical parameters such as lung collagen hydroxyproline, hexosamines

and uronic acids that are markers of neoplastic development reduce significantly (P <0.001) in the treated animals as compared to untreated control animals. The treatment also reduces serum γ-glutamyltranspeptidase (γ-GT) and sialic acid levels as compared to the control animals [46].

c. Lymphoma

***In vivo* studies**

Singh et al. (2005) have reported differentiation and antitumor functions of tumor-associated macrophages (TAM) derived dendritic cells (DC) obtained from tumor-bearing host administered with alcoholic extract of TC [47]. Singh et al. (2006) have also investigated the effect of *in vivo* administration of alcoholic extract of whole plant of TC on the proliferation and myeloid differentiation of bone marrow hematopoietic precursor cells in mice bearing a transplantable T cell lymphoma of spontaneous origin designated as Dalton's lymphoma (DL). The study has indicated that TC can influence the myeloid differentiation of bone marrow progenitor cells and the recruitment of macrophages in response to tumor growth in situ [48].

d. Skin Cancer

***In vitro* studies**

Metastasis is also a major problem of treatment failure in cancer patients. Administration of the polysaccharide fraction of TC has been found to be very effective in reducing the metastatic potential of B16F-10 (murine melanoma cells) [46].

e. Breast cancer

***In vitro* studies**

Aqueous and methanolic fractions of TC stem have been found to exhibit potent cytotoxic activity against breast cancer cell lines T-47D, MCF-7 and MDA-MB-231 [46, 49].

***In vivo* studies**

A prospective, randomized, double blind placebo controlled clinical trial has been conducted on breast cancer patients. Consenting breast cancer patients, who were receiving adjuvant therapy (CMF regimen), were recruited and randomized to drug and placebo group. From the results, it appears that TC provides some protection against the cancer chemotherapy induced leucopenia [50].

f. Prostate cancer

***In vitro* studies**

Aqueous fraction of TC stem has also been demonstrated to exhibit potent cytotoxic activity against prostate cancer cell line PC-3 [45].

g. Oral cancer

***In vitro* studies**

Cancer of the oral cavity is a common disease in Asian countries and oral squamous cell carcinoma (OSCC) is clinically the most common in Indian males. TC extracts have been shown to inhibit cell proliferation and induce cell death in a dose-dependent (25-75 µg/ml) and time dependent (24-120 h) manner in oral squamous cell carcinoma cell line along with a significant cytostatic effect *in vitro* [47].

h. Cervical cancer

***In vitro* studies**

Jagetia et al. (1998) have found that TC effectively kills HeLa cells *in vitro* and thus it indicates that TC needs attention as an anti-neoplastic agent [51-53]. In the study, exposure of HeLa cells to 0, 5, 10, 25, 50 and 100 mg/ml of TC extract (methanol, aqueous and methylene chloride) resulted in a dose dependent but significant increase in cell killing when compared to non-drug treated controls [51].

i. Brain Tumor

***In vivo* studies**

Misra and Kaur (2013) have investigated the anti-brain cancer potential of 50% ethanolic extract of TC using C6 glioma cells. TC ethanolic extract has been found to significantly reduce cell proliferation in dose-dependent manner and induce differentiation in C6 glioma cells, resulting in astrocyte-like morphology. Reduced proliferation of cells is accompanied by enhanced expression of senescence marker mortalin and its translocation from perinuclear to pancytoplasmic spaces. Further, TC ethanolic extract also shows anti-migratory and anti-invasive potential as depicted by wound scratch assay and reduced expression of plasticity markers such as neural cell adhesion molecule (NCAM) and polysialylated-neural cell adhesion molecule (PSA-NCAM) along with matrix metalloproteinase (MMP-2 and 9). On analysis of the cell cycle and apoptotic markers, TC ethanolic extract treatment has been shown to arrest the C6 cells in G0/G1 and G2/M phase, suppressing expression of G1/S phase specific protein cyclin D1 and anti-apoptotic B-cell lymphoma-extra large protein (Bcl-xL), thus supporting its anti-proliferative and apoptosis inducing potential. This suggests that TC ethanolic extract and its active components may prove to be promising phytotherapeutic interventions in gliobalstoma multiformae [54].

j. Experimental Tumors (Ehrlich ascites carcinoma)

Thippeswamy and Salimath (2007) have reported the mechanism of cell death exhibited by the hexane extract fraction of TC against Ehrlich ascites tumor in mice proving that the hexane fraction of TC is capable of inducing apoptosis in the tumor cells *in vivo* [55].

Curcuma longa (CL)

Curcuma longa Linn (CL), also known as turmeric and 'Haldi' in Hindi is an Indian spice and medicinal plant belonging to *Zingiberaceae* family [Fig-5] and is extensively used in Ayurveda, Unani and Siddha medicine and as a home remedy for various diseases and is a known 'wound-healer'. Turmeric/curcumin also acts as a potent anti-carcinogenic compound and was recently nominated by the National Cancer Institute for study [56]. The active component curcumin induces apoptosis, inhibits cell cycle progression and prevents cancerous cell growth. The mechanism responsible for apoptosis involves inhibition of cell signaling pathway genes like protein kinase B/Akt, nuclear factor kappa-light chain-enhancer of activated B cell (NF-KB), activator protein 1 transcription factor (AP1) and DNA damage. Curcumin's inhibitory effect on carcinogenesis has been demonstrated in several animal models of various tumor types including oral cancer, mammary carcinoma and intestinal tumors [57-59].

(a)

(b)

(b)

(d)

(e)

Fig-5 (a) *Curcuma Longa* (L.) Habit (b) Leaves (c) Flower (d) Fruits (e) Rhizome.

Image courtesy naturesalive.wordpress.com, www.nmpb.nic.in and www.flowersofindia.net.

Classification of *Curcuma longa*

Kingdom: Plantae
Division: Tracheophyta
Class: Liliopsida
Order: Zingiberales
Family: Zingiberaceae
Genus: Curcuma
Species: *longa*

Active ingredients: The active constituent of turmeric is curcumin [Fig-6], a flavonoid, responsible for the yellow color and volatile oils like tumerone, atiantone and zingiberone.

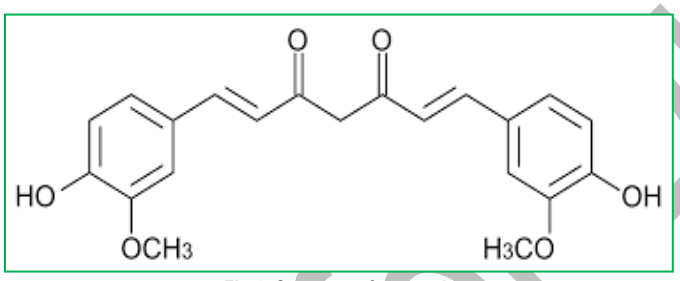


Fig-6 Structure of curcumin

Image courtesy www.en.wikepedia.org

Anticancer activity of CL:

a. Head and Neck Squamous Cell Carcinoma

***In vitro* studies**

Studies of curcumin in various head and neck cancer cell lines *viz.*, CCL23 (human laryngeal cell line derived via HeLa contaminant), CAL27 (squamous cell carcinoma epithelial cell line derived from tongue), UM-SCC14A and UMSCC1 (human oral, upper aero-digestive tract, head and neck squamous cell carcinoma cell line) [60-63], have demonstrated decreased cell growth and survival, concomitant with the compound's effects on molecular pathways involved in cellular proliferation. Expression of constitutively active NF-ĸB and inhibitor kappa B kinase (IKK) has been observed in multiple oral squamous cell carcinoma cell lines, and curcumin treatment was shown to suppress growth and survival of these cell lines via inhibition of NF-KB activation [64].

***In vivo* studies**

Curcumin has demonstrated *in vivo* growth suppressive effects on head and neck squamous cell carcinoma using nude mouse xenograft models. The lipophilic nature of curcumin and relative insolubility in aqueous solutions, combined with short half-life and low bioavailability following oral administration has presented a significant challenge in developing an effective delivery system for its use as a chemotherapeutic agent [65]. In an effort to overcome this obstacle, various strategies are being tried including the use of piperine as an adjuvant agent to slow curcumin breakdown as well as the development of liposomal, phospholipid and nanoparticulated formulations of the compound to enable intravenous administration [65].

b. Colon cancer

***In vitro* studies**

The ethanolic extract of CL rhizomes has been studied using a number of colon cancer cell lines *viz.* colon 502713, and Colo-205 [66]. Curcumin I, curcumin II (monodemethoxycurcumin) and curcumin III (bisdemethoxycurcumin) from CL have also been assayed for their cytotoxicity, antioxidant and anti-inflammatory activities. These compounds showed good activity against colon cancer [67, 68].

Epidemiological studies attribute the low incidence of colon cancer in India to the chemopreventive and antioxidant properties of diets rich in curcumin [69].

***In vivo* studies**

Liposomal formulations of curcumin have been studied in various cancers including colorectal [70].

c. Leukemia

***In vitro* studies**

Curcumin has been shown to induce apoptosis among leukemia B lymphoma cells and inhibits the multiplication of leukemia cells in laboratory studies. Hashim et al. (2013) have evaluated the anticancer effect of ethanolic extracts of CL against two human leukemic cell lines *viz.* U937 (human monocytic leukemia cell line) and MOLT-4. Results have shown that ethanolic extract of CL induced apoptosis in both these cell lines [71]. Curcumin has also been shown to cause apoptosis in HL60 (human promyelocytic leukemia cell line) by induction of caspases 8 and 9 [72]. Curcumin also efficiently induces apoptosis in K562 (human chronic myelogenous leukemia cell line) [73].

d. Renal Cancer

Curcumin has been shown to cause induction of apoptosis in Caki (human kidney carcinoma cells) by activation of Akt dephosphorylation, B-cell lymphoma 2 (Bcl-2), Bcl-xL and inhibitor of apoptosis (IAP) protein inhibition, as well as cytochrome c release and caspase 3 activation [74].

e. Prostate Cancer

The effect of curcumin has also been studied on PC-5 (human prostate cancer cell line) and was found to be growth inhibitory [66, 75].

f. Breast cancer

***In vitro* studies**

Curcumin has been found to induce apoptosis in MCF-7 cell line. The efficacy of curcumin alone and in combination with tamoxifen has been investigated in the established antiestrogen-resistant breast cancer cell lines MCF-7/LCC2 and MCF-7/LCC9. Curcumin treatment displays anti-proliferative and pro-apoptotic activities and induces cell cycle arrest at G2/M phase. Moreover, the combination of curcumin and tamoxifen has been found to result in a synergistic survival inhibition in MCF-7/LCC2 and MCF-7/LCC9 cells. Curcumin targets multiple signals involved in growth maintenance and resistance acquisition in endocrine resistant cells. These findings suggested that curcumin alone and combinations of curcumin with endocrine therapy may be of therapeutic benefit for endocrine-resistant breast cancer [76].

g. Lung Cancer

***In vitro* studies**

Curcumin also leads to apoptosis in scleroderma lung fibroblasts (SLF) without affecting normal lung fibroblasts (NLF) [77]. This effect seems to be due to the weak level of protein kinase (PK) C3 in SLF, generating low levels of GST. Cytotoxic effects of ethanolic extract of curcumin have also been studied on A-549 cell line. The cytotoxic activity of the extract was found to 18% against the tested cell line [66].

h. Liver Cancer

***In vitro* studies**

Studies reporting anticancer activity of curcumin and ethanolic extract of turmeric *in vitro* (human hepatocellular carcinoma cell line) have revealed that both curcumin and the crude ethanolic extract have great potential in the prevention and cure of hepatic cancer [78]. The effects of *curcuma* oil on Hepa1-6 (mouse hepatoma cell line) have been evaluated. *Curcuma* oil has been found to inhibit cell growth and induces cell death in Hepa1-6 cells [79].

***In vivo* studies**

It has been reported that treatment with *curcuma* oil can decrease the incidence of hepatocellular carcinoma (HCC). *Curcuma* oil has been found to protect mice with hepatic injury from inflammatory and oxidative stress and can inhibit hepatoma cell growth *in vivo* [80].

i. Skin Cancer

The antiproliferative and pro-apoptotic effects of curcumin have been studied in melanoma [61, 81]. Another study has reported that curcumin can protect animals from the tumor-producing effects of deadly gamma radiation and it protects against damaging ultraviolet light, which is known to play a role in the development of skin cancer [82, 83]. Curcumin has also been reported to induce apoptosis in human melanoma cells through a Fas receptor/caspase-8 pathway independent of p53 [84].

j. Ovarian Cancer

The effect of curcumin has been studied on OVCAR-5 (human ovarian carcinoma cell line) [66, 85, 86]. Ayurveda especially recommends turmeric for cancers of the female reproductive system, namely breast and uterine cancer. Anticancer activity of the rhizomes of turmeric has been evaluated on CHO cells (Chinese hamster ovary cell line). Turmeric extract inhibited the cell growth in CHO cells at a concentration of 0.4 mg/ml. The cytotoxic effect was found within 30 min at room temperature [86].

k. Oral Cancer

***In vitro* studies**

Chakravarti et al (2010) have demonstrated that curcumin suppressed the growth of immortalized oral mucosal epithelial cells and squamous cell carcinoma cells (UMSCC22B and SCC4) while having minimal effect on normal oral epithelial cells [87, 88]. Curcumin has been shown to reduce the efficiency of the eIF4F translational complex of these immortalized cells via suppression of phosphorylation of eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1), eukaryotic translation initiation factor 4 gamma (eIF4G), eukaryotic translation initiation factor 4B (eIF4B) and CAM kinase (Mnk1), as well as a reduction in the total levels of eIF4E and Mnk1. The effect of ethanolic extract of CL has been studied on Hep-2 (Human epithelial type 2 human laryngeal carcinoma cell Line) [89]. Results have shown that the crude extract of C. *longa* displays significant cytotoxic against Hep-2 cell line in a concentration and time dependent manner [89, 90].

***In vivo* studies**

In SAS oral cancer cells, curcumin has been found to induce the promoter activity of insulin-like growth factor binding protein-5 (IGFBP-5) and CCAAT/enhancer-binding protein alpha (C/EBPalpha), proteins involved in the suppression of head and neck cancers. The inhibitory effects of curcumin on IGFBP-5 and C/EBP-alpha were mediated via p38 activation, and resulted in decreased *in vivo* tumorigenesis in a mouse xenograft model [91].

l. Pancreatic Cancer

***In vivo* studies**

Liposomal formulations of curcumin have been studied in various cancers including pancreatic, cancer colorectal and prostate [92].

m. Cervical Cancer

Curcumin has also been reported to cause induction of apoptosis in HeLa (human cervical adenocarcinoma cell line) [72].

n. Brain Tumor

***In vitro* studies**

Ethanolic extract of curcumin has also been studied on SF-295 (human glioblastoma non-epithelial cell line). However, no activity has been reported against this cell line [66].

o. Experimental Tumors (Dalton's ascites lymphoma)

Both turmeric extract and curcumin have been shown to be cytotoxic to Dalton's lymphoma cells grown as ascites form [93].

p .Animal cell lines

Cytotoxic activity of crude ethanolic extract of *Curcuma longa* rhizome has also been evaluated on two malignant cell lines *viz.* murine mammary adenocarcinoma (AMN-3) cell line and one transformed cell line of rat embryogenic fibroblast (REF-3) [90]

Discussion

WS is one of the most important herbs of Ayurveda (the traditional system of medicine in India) used since millennia for its wide-ranging health benefits. It is an ingredient in many formulations prescribed for a variety of musculoskeletal conditions (e.g., arthritis, rheumatism), and as a general tonic to increase energy, improve overall health and longevity, and prevent disease in athletes, the elderly, and during pregnancy [94,95]. WS is well known for its other biological activities like adaptogenic/anti-stress [96-98], immunomodulatory [99,100], anti-ageing [96-98, 100, 101], anti-fatigue [96-98, 102], antioxidant [103], anti-parkinsonism [104,105], anti-ulcerogenic [97, 98], anti-tumors/adenomas [106, 107], and support healthy thyroid function [108]. The results of the studies described above demonstrate that WS and its chemical ingredients are effective in prevention and treatment of different kinds of cancer like colon cancer, lung cancer, blood cancer, skin cancer, breast cancer, renal cancer, fibrosarcoma, prostate cancer and pancreatic cancer. At the International Institute of Herbal Medicine (IIHM), Lucknow, clinical studies are being conducted to prove the efficacy of WS in prevention and treatment of different forms of cancer including prostate, dermatofibrosarcoma, breast cancer, fibroids of uterus, squamous cell carcinoma of penis etc. especially in last stages, and this wonder medicinal herb has been found to be beneficial in many patients [109]. Infact, some cases of lung cancer who have been refused modern therapy, have recovered clinically and radiologically with ashwagandha therapy [110]. Clinical studies suggest its use as anti-tumor and immunomodulatory agent in sarcoma, brain cancer, uterine tumor, fibroids and other tumors including endodermal carcinoma [109]. Besides having anticancer activity, it may also reduce the side effects of anticancer agents which invariably make the patient's life miserable and reduce immunity.

TC has also been demonstrated to possess anti-cancer activity, this activity has been mostly shown in animal models. Root extract of TC has been shown to possess radio protective role. Dichloromethane extracts of TC show cytotoxic effects owing to lipid peroxidation and release of LDH and decline in GST in Ehrlich ascites carcinoma and cultured HeLa cells [111, 112]. In pre-irradiated mice, root extract has been shown to significantly affect radiation-induced rise in lipid peroxidation and decline of GSH in testes [113]. Most of the synthetic chemotherapeutic agents have been found to cause toxic side effects on the living organisms [114]. The effect of TC has been reported better than doxorubicin treatment [51].

A capsule containing WS and TC in equal proportions has been formulated [115]. The designed formulation is currently being tested in clinical studies for its efficacy [115]. The chemoprotective effect of combined herbal extract of *Withania somnifera*, *Ocimum sanctum* (Tulsi) and TC have been studied in the chemotherapy of cancer. 50% ethanolic extract of the herbs has been tested on blood and *in vitro* cytogenic analysis of the cells at metaphase showed chromosomal aberrations. It has been concluded that a combination of plant extract of *Ocimum sanctum*, WS and TC when given along with chemotherapy, shows decreased chromosomal aberrations [116].

CL again is a promising anticancer agent. Turmeric, a dried powder derived from the rhizome of CL, has been used for centuries in certain parts of the world and has been linked to numerous biological activities including antioxidant, anti-inflammatory, anticancer, antigrowth, anti-arthritic, anti-atherosclerotic, antidepressant, anti-aging, antidiabetic, antimicrobial, wound healing, and memory-enhancing activities. One component of turmeric is curcumin, which has been extensively studied, as indicated by more than 5600 citations, most of which have appeared within the past decade. Recent research has identified numerous chemical entities from turmeric other than curcumin.

It is unclear whether all of the activities ascribed to turmeric are due to curcumin or whether other compounds in turmeric can manifest these activities uniquely, additively, or synergistically with curcumin. However, studies have indicated that turmeric oil, present in turmeric, can enhance the bioavailability of curcumin. Studies over the past decade have indicated that curcumin-free turmeric (CFT) components possess numerous biological activities including anti-inflammatory, anticancer and antidiabetic. Elemene derived from turmeric is approved in China for the treatment of cancer. The CFT components include turmerin, turmerone, elemene, furanodiene, curdione, bisacurone, cyclocurcumin, calebin A, and germacrone [117].

The ability of curcumin to induce apoptosis in cancer cells without cytotoxic effects on healthy cells contributes to the understanding of the anti-cancer potential of curcumin. The mechanisms by which curcumin exerts its anti-cancer effects are comprehensive and diverse, targeting many levels of regulation in the processes of cellular growth and apoptosis. Besides the vertical effects of curcumin on various transcription factors, oncogenes and signaling proteins, it also acts at various temporal stages of carcinogenesis leading to DNA mutations through the process of tumorigenesis, growth and metastasis. Because of the far-reaching effects and multiple targets of curcumin on the cell growth regulatory processes, it holds much promise as a potential chemotherapeutic agent for many human cancers.

Curcumin inhibits the enzyme topoisomerase, which is required for the replication of cancer cells. It strongly inhibits DNA and RNA synthesis and increases mitochondrial membrane permeability; a very significant property in the apoptosis of proliferating cells. It can also prevent proliferation by cell cycle arrest in the G2/M phase in a variety of malignant tumors [56]. G2/M arrest renders cells more susceptible to the cytotoxic effects of radiation, suggesting that curcumin may find significance as a radio sensitizer [118]. The ability to inhibit cyclooxygenase 2 (COX-2) gene over expression, which is implicated in the carcinogenesis of many different tumors, has suggested a plausible role of curcumin to protect children against leukemia.

Earlier research conducted at the University of Texas, M.D. Anderson Cancer Centre has shown that curcumin can also inhibit cytochrome P450, a phase I metabolizing enzyme, which is required for toxic chemicals such as heterocyclic amines to induce DNA adduct formation leading to carcinogenesis [119] and on the other hand to induce phase II metabolizing enzymes generally regarded as favorable detoxifiers, implies its strong promise as a possible safe and nontoxic chemo preventive and/or treatment agent for colon, skin, stomach, liver, lung, duodenum, soft palate and breasts cancers [87, 120]. Furthermore, curcumin can enhance sensitivity of cancer cells to certain drugs commonly used to combat cancer and can potentially improve the effectiveness of radiation treatment.

While studies of curcumin as a single agent in the treatment of head and neck cancer have shown promising results, there is significant interest in potentially using the compound as an adjuvant agent in combination with standard platinum-based chemotherapy for the treatment of tumors. Data in CAL27 and UMSCC-1 cell lines have demonstrated an increased growth suppressive effect in cells treated with a combination of liposomal curcumin and cisplatin, both *in vitro* as well as in mouse xenograft tumor models [120]. While treatment with either curcumin or cisplatin *in vitro* resulted in cell death, a combination of curcumin and suboptimal concentrations of cisplatin demonstrated a significant growth suppressive effect compared to treatment with either agent alone.

Alcoholic extracts of WS, TC and CL along with other plant extracts have been used in a herbal formulation as memory enhancer for Alzheimer's disease [121] and also as oil formulations for treatment of rheumatic diseases.

Even if one goes the allopathic route to treat their cancer, they can still use turmeric to increase the effectiveness and decrease some of the side effects of cancer treatments. The efficacy of turmeric to decrease cell viability, cell cycle arrest and induction of apoptosis is encouraging to the development of a natural drug with known Nuke-B inhibitory activity [122]. In short, turmeric is an example of a natural dietary agent capable of acting at multi levels in cellular pathways for the prevention or treatment of diseases with multifactorial etiologies such as colon, skin, stomach, liver, lung duodenum, soft palate and breast cancer [123].

Conclusion

Plants have been used for treating diseases since time immemorial. The aim of the present review was to highlight recent research findings on the anti-carcinogenic property of medicinal plants such as *Withania somnifera* (ashwagandha), *Tinospora cordifolia*, (guduchi), and *Curcuma longa* (haldi), which find ample mention for their medicinal properties in Ayurveda, and can be, as such, utilized in various herbal formulations as complementary medicine in the management and treatment of cancer. It is no longer an option to ignore ayurvedic drugs or treat them as something unconventional from regular medical practices. Future research in this area would help to identify safe and effective anticancer drugs and would also enhance the exploration of their mechanism of action.

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