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Potential of *Hygrophila auriculata* (Schumach.) Heine as a source of future anti-cancer drugs: A comprehensive review

Sudeshna Saha and Santanu Paul

Abstract
Conventional treatments have varying degrees of success in ameliorating cancer. But administration of chemotherapeutic drugs can often cause side effects in afflicted individuals, exacerbating the morbidity and mortality associated with this disease. Efforts are being made all over the world to develop new and better anti-cancer drugs from the vast repertoire of plant-derived phytoconstituents available in nature. At least four groups of plant-derived annti-neoplastic drugs are already available commercially. In this review, the potential of the angiospermic plant *Hygrophila auriculata* (Schumach.) Heine as a source of anti-cancer drug has been discussed. The crude extract of the plant has shown anti-tumour efficacy in Ehrlich ascites carcinoma and Sarcoma-180, MDA-MB-435S and hepatocarcinogenesis in male Wistar rats. Five phytoconstituents of *H. auriculata* have been reported to possess extensive anti-cancer potential and exhibit anti-tumour effects in many different cell lines and in many different types of cancer.

Keywords: *Hygrophila auriculata*, Phytochemicals, Anti-cancer drug developemnt

1. Introduction
Cancer, one of the most dreaded diseases of the 21st century, is a generic term referring to a large group of non-communicable diseases that can affect any organ or tissue system of the body in both young and old individuals. It is characterized by abnormally dividing cells with "limitless replicative potential". These cells have the capacity to metastasise to other healthy parts of the body and cause secondary tumours [1]. Carcinogenesis is a multi-step process, where several malfunctions in genetic and epigenetic controls ultimately add up to transform normal cells into malignant tumours [2]. Cancer maybe caused due to poor lifestyle choices (tobacco use, sedentary lifestyle with lack of physical activities, alcohol abuse, decreased consumption of fruits and vegetables, obesity etc.), viral infections such as hepatitis B (HBV), hepatitis C (HCV) and human papilloma virus (HPV) [3], environmental pollutions, ionizing radiation [4], or by inheritance of genetic defects [5].
Cancer is one of the leading causes of worldwide mortality, with approximately 14 million new cases in 2012 [6] and 8.8 million cancer deaths in 2015 [3]. The total number of annual cancer cases is expected to rise by 70 percent over the next two decades. Globally, the most common types of cancers are cancers of lung (1.69 million deaths), liver (788,000 deaths), colorectal (774,000 deaths), stomach (754,000 deaths), and breast (571,000 deaths) [3]. In 2012, the 5 most common sites of cancer diagnosis were lung, prostate, colorectum, stomach and liver in men, and breast, colorectum, lung, cervix and stomach in women. In 2012, about 165,000 children under 15 years of age were diagnosed with cancer [7].
Several conventional cancer treatment options are available, such as surgery, radiation therapy, chemotherapy, targeted therapy, accompanied by palliative care etc. which have varying degrees of success. However these treatments are usually accompanied by a host of detrimental side effects that can further weaken the afflicted individuals. For instance, some well-known chemotherapeutic drugs can cause toxicity in healthy tissues and organs [8]. The well-known chemotherapeutic agent 5-fluorouracil (used mainly for gastro-intestinal cancer) can cause myelosuppression, diarrhoea [9], cardiotoxicity [10] and exhibit vasospastic toxicity in rare cases [11]. Similarly doxorubicin, another widely used chemotherapeutic drug has been noted to cause cardiac toxicity [12, 13] renal toxicity [14] and myelosuppression [15].
In the light of several adverse effects arising out of the use of the common anti-tumour drugs, natural products obtained from plants have been investigated as an alternative source of therapeutic agents which potentially might have lesser side effects.

The concept of plant-derived anti-cancer drugs is not new, and at least four classes of drugs are already available commercially in the market nowadays- the vinca alkaloids (vinblastine, vincristine and vindesine) from *Catharanthus rosues* (L.) G. Don, the epipodophyllotoxins (etoposide and teniposide) from *Podophyllum peltatum* L., the taxanes (paclitaxel and docetaxel) from *Taxus brevifolia* Nutt. and the camptothecin derivatives (camptotecin and irinotecan) from *Camptotheca acuminata* Decne.[8]. Additionally, the anti-cancer efficacies of many other plants are also being investigated. Several plants such as *Tinosporia sinensis* (Lour.) Merr. [16], *Andrographis paniculata* (Burm. F.) Nees (possess the diterpene lactone andrographolide which shows cytotoxic activity against a variety of cancer cells) [17], *Centella asiatica* (L.) Urb. [18], *Curcuma longa* L. (possesses curcumin which is known to inhibit the proliferation of a number of tumour cell types) [19, 20] etc. have shown significant promise in this area.

Hence, it appears that in future many new chemotherapeutic agents might originate from the plant kingdom. In this context, potential of the angiospermic plant *Hygrophila auriculata* (Schumach.) Heine (which has shown some anti-cancer effects) as a source of new anti-tumour drugs has been investigated and discussed in this review.

2. Traditional uses and pharmacological effects of *H. auriculata*

H. auriculata (Family-Acanthaceae, synonyms- *Asteracantha auriculata* Nees, *Asteracantha longifolia* Nees, *Hygrophila schulli* M.R. Almeida and S.M. Almeida, *Hygrophila spinosa* T. Anderson etc.) [21] commonly known as the "Marsh Barbel" in English, "Kokilaksha and "Ikshura" in Sanskrit, "Talmakhana" and "Kamtakalya" in Hindi [22] and "Kulekhara" or "Kuliakhara" in Bengali, is a perennial, often aquatic or hygrophilous herb [23] and is widely known for its multifarious medicinal uses.

Fig 1: Photograph showing leaves and flower of *H. auriculata*

2.1 Traditional (ethnomedicinal) uses

This plant has been traditionally used in the preparation of several Ayurvedic medicines such as Aviltholadi Bhasmam (treatment of disorders of liver, spleen, oedema etc.) Yakrut Shula Vinashini Vatika (treatment of liver and spleen disorders), Panaviraladi Bhasmam (treatment of liver and spleen disorders, oedema, abdominal distension) etc. [22]. A review on the phytochemical constituents of *H. auriculata* by Patra *et al.*, 2009 [24] noted that plant organs like roots, leaves, seeds, flowers, fruits, even the whole plant and plant ashes have been reported to have been used in the treatment of various diseases [25, 26, 27, 28].

2.2 Pharmacological Effects

Several pharmacological activities have also been reported from *H. auriculata*. The plant has been reported to have analgesic effect, anti-motility effect [29], anti-bacterial effect, anthelminthic effect [30], anti-diabetic effect [31] and relieving diabetic neuropathy [32], anti-inflammatory effect [33, 34], anti-pyretic effect [34], diuretic effect [35], haematopoetic effect [36, 37, 38], hepatoprotective effect [39-45], nephroprotective effect [46, 47, 48], neuroprotective effect[49], effect on sexual characters [50, 51], and cytotoxic effect [52]. Crude extract has also been noted for its anti-oxidant potential [31, 33, 40, 42, 49]. The different pharmacological activities reported from this plant have been diagrammatically presented in Fig 2.

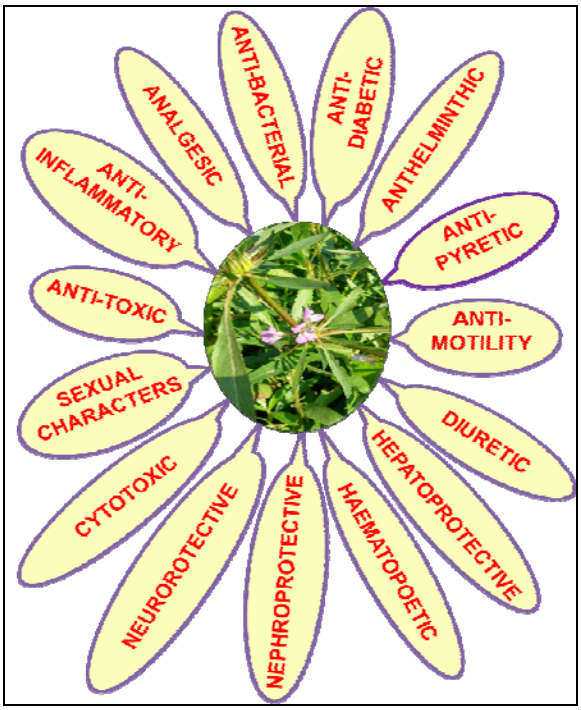

Fig 2: Pharmacological effects of *Hygrophila auriculata* (Schumach.) Heine

3. *H. auriculata* and cancer

3.1 Effects of crude extracts on cancer

The crude extract has shown some promise as anti-cancer agent, as briefly described in the following section.

The processed petroleum ether extract exhibited anti-tumour activity on Ehrlich ascites carcinoma (a type of murine adenocarcinoma) and Sarcoma-180 (referred to as EAC/S-180) bearing mice. The extract could successfully decrease packed cell volume by 50% and the tumour fluid volume by the end of 3 weeks. It also increased the lifespan of tumour bearing mice [53].

In another study, out of 16 Bangladeshi medicinal plants investigated for their cytotoxic uses, the methanolic extract of seeds of *H. auriculata* showed no toxicity against healthy mouse fibroblasts (NIH3T3), but selective cytotoxicity against breast cancer cells (MDA-MB-435S) with IC_{50} of 1.58 mg/mL. The aqueous extract of seed displayed selective toxicity against colon cancer cell line HT-29 with an IC_{50} of 0.22 mg/mL. The result of this study indicated the possibility that the methanolic extract of seeds possess selective cytotoxicity against cancerous cells instead of healthy cells [54].

The chemopreventive efficacy of the methanolic extract against experimentally induced hepatocarcinogenesis has also been noted in male Wistar rats. The methanolic extract may exert chemopreventive activity by inhibiting the formation of DNA adduct with carcinogen 2-acetylaminofluorene [55].

Thus, the crude extracts (chloroform, methanolic and aqueous) showed anti-tumour and chemoprotective effects.

3.2 Discussion of anticancer efficacies of the phytoconstituents on various *in vitro* and *in vivo* systems

Any living organism, whether it be a microbe, plant or fungi, shows pharmacological effects due to the activity of a single or multiple bioactive compounds; these compounds are integral components of the chemical make-up of the organism. Plants are specially noted for their secondary metabolites, which are usually unique to a particular plant or group of plants. The pharmacological activities of plants are often attributed to the secondary metabolites.

A number of compounds (including secondary metabolites) such as flavonoids, alkaloids, triterpenes, aliphatic esters, sterols, etc. have been reported from this plant. According to Daniel, 2005 flavonoid apigenin (with derivatives apigenin-7-o-glucuronide and 7-o-glucoside) occurred in leaves and flowers, while Balraj *et al.*, 1982 reported another flavonoid luteolin (and derivative luteolin-7-rutinoside) from leaves. Balraj *et al.*, reported alkaloids asteracanthine and asteracanthicine from seeds. Daniel, 2005 also reported the triterpene lupeol; the triterpenes betulin and hentriacontane were reported from different plant parts by Sharma *et al.*, 2002 and Chopra *et al.*, 1958 respectively. Dewanji *et al.*, 1997 reported the occurrence of β-carotene. Shailajan *et al.*, 2008 and Sharma *et al.*, reported sterols β-sitosterol and stigmasterol. A number of authors also reported the presence of primary metabolites such as carbohydrates maltose, xylose, rhamnose etc., fatty acids such as myristic acid, palmitic acid, stearic acid, etc., vitamins ascorbic acid, nicotinic acid, amino acids histidine, phenylalanine, lysine etc. Additionally, some other compounds such as vannilic acid, syringic acid, n-triacontane etc. [24]. The various phytoconstituents reported till now have been represented by Fig 3.

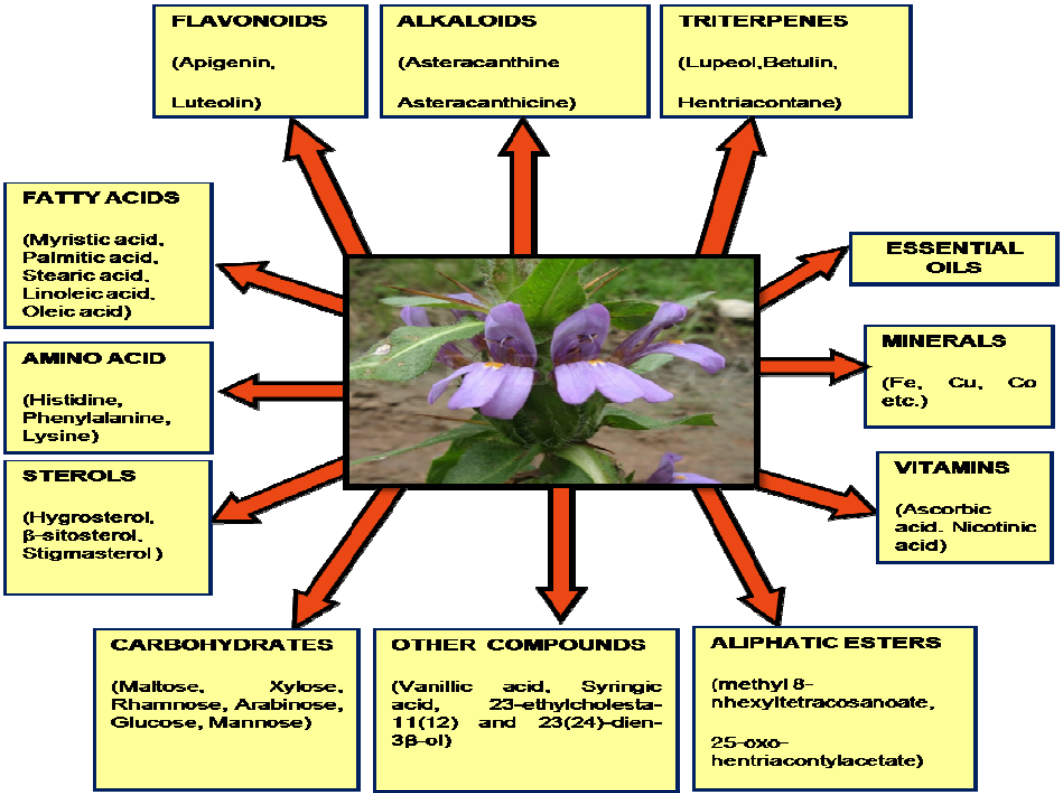

Fig 3: Phytochemicals reported from *Hygrophila auriculata* (Schumach.) Heine

Among the phytochemicals, compounds apigenin, luteolin, lupeol, betulin, lupeol and β-sitosterol have already been noted for their anti-tumour potential, as evident by the vast repertoire of information available in literature. In contrast, stigmasterol has fewer reports regarding its anti-cancer efficacies. The constituents with anti-cancer potential are

represented in figure 3.

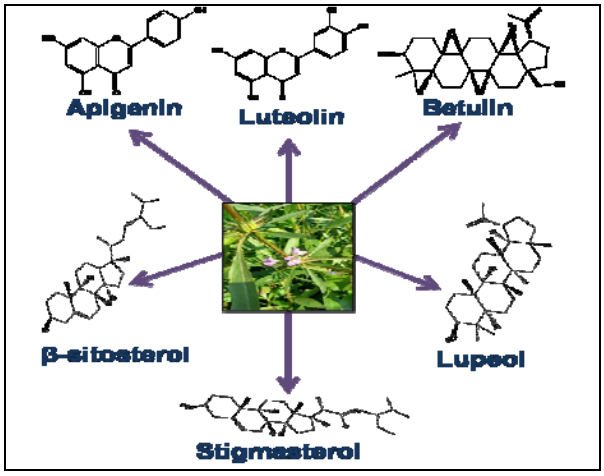

Fig 4: Phytochemicals having potent anti-cancer properties reported from *Hygrophila auriculata* (Schumach.) Heine

The anti-tumour potential of these compounds are discussed as follows.

3.2.1 Apigenin

Chemically apigenin is 4', 5, 7-Trihydroxyflavone, also known as Chamomile, Apigenol etc. The molecular formula is $C_{15}H_{10}O_5$ with molecular weight of 270.24 g/mol [56]. In nature apigenin has been reported to occur as a dimer called biapigenin, mainly isolated from the buds and flowers of *Hypericum perforatum* L.**,** grapefruits, onions, oranges, leafy vegetables like parsley, beer, wine etc. [57]. Apigenin is a component of chamomile, prepared from the dried flowers of *Matricaria chamomilla* L. [58]. In natural sources, apigenin can occur as apigenin-7-*O*-glucoside and also as several other acylated derivatives [59].

A list of cancers and cancer cell lines whose growth has been inhibited by apigenin has been given in table 1.

Table 1: List of different types of cancers and cancer cell lines whose growth and progression has been inhibited by apigenin

Organ affected	Name of cancer	Cell line affected	Reference(s)
Blood	Acute T-cell leukemia	Jurkat T	[60]
	Histiocytic leukemia	U937	[61]
	Acute promyelocytic leukemia	HL-60	[62]
Bone	Osteosarcoma	MG-63	[63]
Breast	Metastatic breast carcinoma	MDA-MB-453	[64, 65]
	Breast adenocarcinoma	MDA-MB-468	[67]
	Breast adenocarcinoma	MCF7	[67, 69]
	Breast adenocarcinoma	SK-BR-3	[68]
	Breast carcinoma	Hs605T	[69]
Cervical	Cervical squamous cell carcinoma	SiHa	[69]
	Cervical adenocarcinoma	HeLa	[70]
Colorectal	Colorectal carcinoma	HCT 116	[71]
	Colorectal adenocarcinoma	HT-29	[63,71,72]
	Dukes' type B, colorectal adenocarcinoma	SW480	[72]
	Colorectal adenocarcinoma	CaCo-2	[72]
Endometrial	Endometrial adenocarcinoma	Ishikawa	[74]
Gastric	Gastric adenocarcinoma	SGC-7901	[75]
Liver	Rat hepatoma	H4IIE	[76]
Neuroblastoma	Stable I-type human neuroblastoma	NUB-7	[77]
	Human neuroblastoma	LAN-5	[77]
	Human neuroblastoma	SK-N-BE(2)	[77]
	Human neuroblastoma	SH-SY5Y	[78]
Ovarian	Ovarian serous cystadenocarcinoma	HO-8910PM	[79]
	Human ovarian carcinoma	A2780	[80, 81]
Pancrease	Pancreatic carcinoma	MiaPaca-2	[82]
	Pancreatic adenocarcinoma	AsPC-1	[82]
	Prostate carcinoma	LNCaP	[83]
	Prostate grade IV adenocarcinoma	PC-3	[83]
	Prostate carcinoma	DUI45	[83]
Skin	Cell type- epidermal keratinocyte	Mouse 308 keratinocyte	[84]
	Mouse melanoma	B16-BL6 injected into mice (*in vivo*)	[85]
Thyroid	Human anaplastic thyroid carcinoma	ARO (UCLA RO-81-A-1)	[86]

1. **Blood cancer**: In Jurkat T cells, apigenin induced apoptosis by activating caspase 3 and inducing cleavage of poly(ADP-ribose) polymerase (PARP). The apoptotic potential of apigenin was correlated with inhibition of proteosomal activity of 20S proteosome and 26S proteosome. Inhibition of proteosomal activity led to accumulation of proteosome target proteins Bax (apoptotic protein) and inhibitor of NFκB-α (inhibitor of NFκB-α prevents the activation of NFκB-α, which is a transcription factor required for cell survival) which led to apotosis induction [60]. In another study, apigenin was one of the flavonoids which could induce apoptosis in U937 cells [62]. Apigenin could also successfully induce apoptosis in HL-60 cells by inducing of release of cytochrome c from mitochondrial membrane, by procaspase 9 processing, by activating caspase 3 and by proteolytic cleavage of poly (ADP) ribose. In this study, out of four different flavonoids studied, apigenin showed highest apoptotic activity [63].
2. **Bone cancer**: Apigenin inhibited the cell cycle

progression of p53 mutant MG-63 cells by inducing G2/M arrest, indicating that apigenin activity might occur in a p53 independent mechanism [63].

3. **Breast cancer**: In MDA-MB-453 cell line, apigenin showed anti-proliferative effect in a dose and time dependent manner [64, 65]. Apoptosis may be induced by both extrinsic and intrinsic pathway, as evidenced by activation of caspase 3, caspase 8 and caspase 9 [65]. The percentage of apoptotic cells was significantly higher in case of combination treatment of apigenin with chemotherapeutic drug 5-fluorouracil, when compared with 5-fluorouracil treatment alone. It is possible that apigenin stimulates MDA-MB-453 cells to respond to 5-fluorouracil treatment by downregulation of ErbB2 and Akt signalling [64]. Apigenin also induced apoptosis in HER2/neu overexpressing cells and suppresses HER2/HER3-PI3K/Akt signalling [66]. Growth inhibition due to cell cycle arrest at G2/M phase occurred in both MCF7 (IC_{50}-7.8µg/mL) and MDA-MB-468(IC_{50}-8.9µg/mL) cell lines, probably by inhibition of cdk1 [67]. In SK-BR-3, cell cycle arrest at G2/M occurred by regulation of cdk1 and p21, while apoptosis occurred via the p53 independent pathway [68]. Combination treatment of apigenin and luteolin with parthenolide exhibited a weak synergestic effect on cell growth of MCF7 and Hs605T [69].
4. **Cervical cancer**: Apigenin inhibited proliferation of HeLa cells by G1 arrest and apoptotic induction, probably by activating p53 [70]. Combination treatment of apigenin and luteolin with parthenolide exhibited a weak synergestic effect on SiHa cell line [69].
5. **Colorectal Cancer**: 5, 6-dichloro-ribifuranosylbenzimidazole and apigenin inhibited CK2 (a serine-threonine kinase reportedly upregulated in most human malignancies) in HCT116 and HT-29; when used in combination with TNF (tumour necrosis factor)-α, a decrease in survival was also observed [71]. Treatment with apigenin also resulted in G2/M arrest in p53 mutant HT-29 cells, indicating that apigenin activity might occur in a p53 independent mechanism [63]. A reversible G2/M arrest was observed in SW480, HT-29 and Caco-2 cell lines, probably by downregulation of cdc2 kinase and cyclin B1[72]. In an *in vivo* study conducted on mouse model, dietary apigenin exhibited anti-tumour effects by reduction of both ornithine decarboxylase (ODC) activity and aberrant crypt foci formation (aberrant crypt foci refers to the preneoplastic lesions of colorectal cancer in both rodents and humans); however clear evidence of cancer prevention was not obtained in this study [73].
6. **Endometrial cancer**: Ishikawa cells treated with phyto-oestrogenic compounds including apigenin resulted in genomic aberrations in the cells, which were identified by array based comparative genomic hybridisation techniques [74]. Appearance of genomic aberrations indicated that apigenin could adversely affect the survival of Ishikawa cells.
7. **Gastric cancer**: In SGC-7901 cells, apigenin inhibited cell growth, cell proliferation and clone formation in a dose-dependent manner. Cells treated with 80 µmol/L apigenin for 48 hours showed distinct changes in morphology in contrast with normal cancer cells and control; the cells became crimpled, the nuclei became broken and the nucleus no longer had smooth boundary [75].
8. **Liver cancer**: In the rat hepatoma cell line H4IIE, non prenylated flavonoid apigenin showed almost no toxicity, while the prenylated flavonoids licoflavone C (8-prenylapigenin) and isobavachin (8-prenylliquiritigenin) showed pronounced toxicity. In this case, apigenin did not show pronounced anti-cancer potential [76].
9. **Neuroblastoma**: Apigenin inhibited growth and induced apoptosis in NUB-7, LAN-5, and SK-N-BE(2) cell lines. Apigenin also exhibited *in vivo* anti-tumour activity by inhibiting NUB-7 xenograft tumor growth in anonobese diabetic/severe combined immunodeficiency mouse model, likely by apoptosis induction. Apoptosis may have been induced by regulating p53-Bax-caspase-3 apoptotic pathway [77]. A decrease in viability of SH-SY5Y cells was also observed due to apigenin treatment. In this case, apoptosis was induced by activation of caspase-3, caspase-9, caspase-12, calpain, release of mitochondrial cytochrome c, intracellular increase of Bax: Bcl-2 ratio etc. [78].
10. **Ovarian cancer**: Apigenin inhibited migration and adhesion of HO-8910PM cells [79]. In A2780 cells apigenin inhibited focal adhesion kinase (a non-receptor protein tyrosine kinase that plays an important role in migration and inhibition of cancer cells) [80]; apigenin suppressed expression of Id1(a DNA binding protein that stimulates cell proliferation, inhibits cell differentiation and facilitates tumour angiogenesis) and thereby inhibited proliferation of A2780 cells [81]. Apigenin also inhibited spontaneous metastasis of ovarian cancer in an *in vivo* study where A2780 cells were implanted in the ovary of nude mice [80].
11. **Pancreatic Cancer**: Combination treatment of gemcitabine (a chemotherapy drug used for treating breast cancer, lung cancer, pancreatic cancer etc.) and apigenin showed growth inhibition and apoptosis induction by down-regulation of NFκB and suppression of Akt activation in MiaPaca-2 and AsPC-1 cell lines [82].
12. **Prostate cancer**: Apigenin induced apoptosis in LNCaP, PC-3, and DU145 cells; apoptosis induction occurred via decrease of Bcl-2 (anti-apoptotic protein) expression, release of mitochondrial cytochrome C, cleavage of caspase-3, caspase-7, caspase-8 and caspase-9 and cleavage of cIAP-2 (an apoptotis inhibitor protein). However, the apoptotic effects were notably reduced in PC-3 and DU145 cells [83].
13. **Skin Cancer**: Over-expression of Cyclooxygenase 2 (COX-2), an isoform of cyclooxygenase (cyclooxygenase is an enzyme associated with conversion of arachidonic acid to prostaglandins) is associated with many different types of cancer, including UVB induced skin cancer. In mouse 308 keratinocyte cell line apigenin reduced COX-2 expression by inhibiting translation via TIAR (T-cell-restricted intracellular antigen 1-related protein, an RNA binding protein) [84]. In an *in vivo* study conducted by injecting B16-BL6 cells into syngeneic mice, it was seen that apigenin induced inhibition of tumour growth without causing toxicity [85].
14. **Thyroid cancer**: Apigenin inhibited proliferation of ARO (UCLA RO-81-A-1) cells by inhibition of both EGFR tyrosine autophosphorylation and phosphorylation of its downstream MAP kinase protein [86].

3.2.2 Luteolin

Chemically luteolin is known as digitoflavone, 3',4',5,7-tetrahydroxyflavone,2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-

4H-chromen-4-one etc. It has a molecular weight of 286.239 g/mol and the molecular formula is $C_{15}H_{10}O_6$ [87]. Luteolin occurs in many fruits, vegetables, and medicinal herbs such as celery, parsley, broccoli, onion leaves, carrots, peppers, cabbages, apple skins, chrysanthemum etc. The Chinese have traditionally used plants rich in luteolin for treatment of various diseases such as hypertension, inflammatory disorders, and cancer [88].

A list of cancers and cancer cell lines whose growth was inhibited by luteolin has been given in table 2.

Table 2: List of different types of cancers and cancer cell lines whose growth and progression was inhibited by luteolin

Organ affected	Name of cancer	Cell line affected	Reference(s)
Bladder	Bladder transitional cell carcinoma	T24	[89]
Blood	Acute promyelocytic leukemia	HL-60	[91]
Breast	Breast adenocarcinoma	MCF-7	[92][93][94][96]
	Breast adenocarcinoma	SK-BR-3	[92]
	Breast adenocarcinoma	MDA-MB-231	[92][96]
Colorectal	Colorectal adenocarcinoma	HT-29	[97, 98]
	Dukes' type C, colorectal adenocarcinoma	HCT-15	[99]
	Colorectal carcinoma	HCT 116 (specifically HCT116-OX)	[101]
	Dukes' type C, colorectal adenocarcinoma	SW620(specifically SW620-OX)	[101]
Gastric	Gastric adenocarcinoma	AGS	[101]
	Gastric adenocarcinoma	SGC-7901	[102]
Liver	Liver epidermoid carcinoma	HLF	[103]
	Hepatocellular carcinoma	HAK-1B (*in vivo*)	[103]
Lung	Large cell lung carcinoma	H460	[104]
	Lung carcinoma	A549	[104, 105, 106]
	Human lung non small cell carcinoma	NCI-H1975	[106]
Oral	Squamous cell carcinoma	SCC-4	[107]
Prostate	Prostate carcinoma	LNCaP	[108]
	Prostate carcinoma	DU145	[108]
	Prostate grade IV adenocarcinoma	PC-3	[108, 109]
Skin	Epidermoid carcinoma	A431	[110]
	Mouse epidermal cell line	JB6P+	[111]

1. **Bladder cancer**: In **T24** cell line, luteolin displayed a dose-dependent inhibition of arylamine N-acetyltransferase (NAT) and NAT1 gene expression [89]. NAT1 was found to be overexpressed in many cancers and was associated with increased cell survival and chemotherapy [90]. Luteolin also inhibited formation of DNA-2aminofluorene adduct formation [89].
2. **Blood cancer**: Luteolin induced apoptosis in HL-60 cells. The IC_{50} after 96 hours was found to be 15±1 μM [91].
3. **Breast cancer**: In the cell lines MCF-7, MDA-MB-231 and SK-BR-3 luteolin reduced cell viability in a dose and time dependent manner. Apoptosis induction occurred by both caspase dependent and independent pathways. Nuclear translocation of apoptosis-inducing factor (AIF) in the cancer cells was probably mediated by activation of ERK and p38 [92]. Luteolin inhibited the stimulatory effect of insulin-like growth factor-1 (IGF-1) on MCF-7 cells, thereby decreasing cell proliferation and inducing apoptosis. IGF-1 can activate mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K), which are two essential pathways for tumorigenesis. The inhibitory effects of luteolin were mediated via the PI3K/Akt pathway [93]. In another study, several genes of the oestrogen signalling pathway(GTF2H2, NCOR1, TAF9, NRAS, NRIP1, POLR2A, DDX5, NCOA3) and genes controlling cell cycle progression (CCNA2, PCNA, CDKN1A, CCND1, PLK1) were regulated by luteolin in MCF-7 cells. Luteolin probably used epigenetic mechanisms to control these genes, such as interactions with type II sites on histone H4 on the gene promoters and regulation of the acetylation states of histone lysine tails [94]. Additionally, it has also been seen that treatment of luteolin in combination with celecoxib (a non-steroidal anti-inflammatory drug [95], specifically COX-2 inhibitor, developed for treating arthritis) showed a synergestic anti-tumour effect on MCF-7 and MDA-MB-231 cells. The combination treatment for 72 hours decreased cell viability and increased apoptosis to a greater extent than when the cells were treated with either apigenin or celecoxib alone [96].
4. **Colorectal cancer**: In HT-29 cells, luteolin inhibited cell proliferation by G1 and G2/M phase arrest. Cell cycle progression was inhibited by decreasing the expression of cyclin B1 and cyclin D1, cdk2, cd4 and cdc2 [97]. Additionally, luteolin also reduced IGF-II secretion and modified the IGF-IR signalling pathway, which may also account for luteolin's ability to suppress cell proliferation in HT-29 cells. Luteolin could also directly inhibit PI3K in a cell free system [98]. Cell proliferation of HCT-15 cells was inhibited by luteolin; this event was concurrent with suppression of non-phosphorylated β-catenin, phosphorylated glycogen synthase kinase-3β (gsk-3β) and cyclin D. Cell cycle arrest at the G2/M phase and apoptosis induction (coressponding with increased activity of bax and caspase 3, decreased expression of Bcl-2) was also observed [99]. The anti-tumour effect of luteolin was also observed in case of oxaliplatin resistant cell lines HCT116 and SW620, designated as HCT116-OX and SW620-OX. Oxaliplatin is a DNA binding third generation platinum chemotherapeutic agent, and can be used as a "first-line treatment for colon cancer". Oxaliplatin exerts its anti-tumour effects by inhibiting DNA synthesis and repair. However, continuous usage over time may confer oxaliplatin resistance to the cancer cells. Luteolin inhibited the Nrf2 pathway (Nrf2-nuclear factor erythroid-2 p45-related factor 2, a transcription factor) *in vitro* and inhibited expression of Nrf2 pathway target genes (NADPH quinine oxidoreductase 1, heme oxygenase-1 and glutathione S-transferase) *in vivo* in the

cells of small intestestine in wild type C57BL6 mice. Malfunction of this pathway may lead to chemoresistance to many cancers; inhibition of the pathway by luteolin reversed the chemoresistance [100].

5. **Gastric cancer**: Luteolin induced apoptosis in AGS cells, associated with increased expression of caspase-3, caspase-6, caspase-9, bax, p53 and decreased expression of Bcl-2 proteins. In AGS cells, luteolin treatment also resulted in downregulation of cdc2, cyclin B1, cdc25C and upregulation of p21 [101]. In SGC-7901 cells, luteolin inhibited cell proliferation and enhanced the sensitization of tumour cells to radiotherapy, with release of mitochondrial cytochrome c, increased expression of caspase-3 and caspase-9 and decreased activity of Bcl-2. [102].
6. **Liver cancer**: Luteolin induced apoptosis in HLF cells *in vitro* and suppressed the growth of HAK-1B hepatoma cells transplanted in nude mice in a time and dose dependent manner. Luteolin targeted STAT3 (signal transducer and activator of transcription 3, an important signalling molecule that has been found to be constitutively active in a number of human tumours) which led to apoptosis induction via increased activity of Fas/CD95 [103].
7. **Lung cancer**: In the cell lines H460 and A549, luteolin induced cell death by both apoptosis and necrosis, associated with superoxide induction leading to activation of c-Jun N-terminal kinase (JNK) [104]. In a different study, it was found that luteolin also induced G1 arrest along with apoptosis in A549 cells. Suppression of cell migration and stress fibre assembly was also observed [105]. In A549 and NCI-H1975 cells, luteolin inhibited hypoxia-induced EMT (EMT-epithelial mesenchymal transition, an important event in cancer metastasis), cell proliferation, motility and adhesion [106].
8. **Oral cancer**: Luteolin reduced the viability of SSC-4 cells and induced apoptosis by activating caspase-3 and caspase-9, and degradation of poly-ADP-ribose polymerase; it also decreased growth of xenograft tumours in mice. Combination treatment of luteolin and paclitaxel increased the SSC-4 cancer cell killing ability of paclitaxel [107].
9. **Prostate cancer**: Luteolin suppressed cell proliferation and induced apoptosis in LNCaP, PC-3 and DU145 cells; androgen receptor (which plays an essential role in prostate tumour formation) was also suppressed by luteolin, which led to inhibition of cell proliferation and apoptosis induction in LNCaP cells [108]. Combination treatment of luteolin and gefitinib (a tyrosine kinase inhibitor for epidermal growth factor receptor) inhibited kinase activity of GAK (cyclin G-associated kinase, involved in clathrin mediated membrane trafficking, often found to be over-expressed in hormone refractory cancer cells; it is also a transcriptional co-activator for androgen receptor) and induced apoptosis in PC-3 cells [109].
10. **Skin Cancer**: Luteolin inhibited cell proliferation and EGFR (epidermal growth factor receptor) tyrosine kinase activity in A431 cells. Suppression of the activity of two metalloproteins, matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9), was also observed. Metalloproteinases are zinc-dependent peptidases that play an important role in cancer metastasis. Apoptotic induction was also observed in A431 cells [110]. Luteolin suppressed UVB induced COX-2 overexpression in JB6P+ cells (mouse epidermal cell line). In case of SKH-1 mouse model, inhibition of COX-2, TNF-α, and PCNA (proliferating cell nuclear antigen) expression in mouse skin was seen. Luteolin treatment suppressed tumour growth and tumour formation *in vivo* as well [111].

3.2.3 Lupeol

The triterpenoid compound lupeol is also known as fagarasterol, clerodol, monogynol B, farganasterol etc. The molecular formula is $C_{30}H_{50}O$ and the molecular weight is 426.729 g/mol [112]. Lupeol has been reported from a number of plants such as white cabbage, pepper, cucumber, tomato, red grapes, *Tamarindus indica* L., *Celastrus paniculatus* Willd., *Bombax ceiba* L. etc. [113].

A list of cancers and cancer cell lines whose growth was inhibited by lupeol has been given in table 3.

Table 3: List of different types of cancers and cancer cell lines whose growth and progression was inhibited by lupeol

Organ affected	Name of cancer	Cell line affected	Reference(s)
Breast	Breast adenocarcinoma	MCF-7	[114]
Colorectal	Dukes' Type C, Colorectal adenocarcinoma	DLD1	[115]
	Colorectal carcinoma	HCT 116	[115]
Gall bladder	Gall bladder carcinoma	GBC-SD	[117]
Liver	Hepatocellular carcinoma	MHCC-LM3	[117]
	Human hepatoma	PLC-8024	[117]
	Human hepatocarcinoma	Huh-7	[117]
	Hepatocellular carcinoma	SMMC7721	[118]
Oral	Head and neck squamous cell carcinoma	TU159	[119]
	Oral cavity squamous cell carcinoma	MDA1986	[119]
Pancreas	Pancreatic adenocarcinoma	AsPC-1	[120]
Prostate	Prostate carcinoma	LAPC4	[122]
	Prostate carcinoma	22Rv1	[122]
	Prostate carcinoma	LNCaP	[122]
	Prostate carcinoma	C4-2b	[122]
	Prostate carcinoma	PC-3	[123]
Skin	Epidermoid carcinoma	A431	[124, 125]
Tongue	Squamous cell carcinoma	CAL27	[119]

1. **Breast Cancer**: The anti-tumour efficacies of lupeol (isolated from the leaves of a medicinal plant *Elephantopus scaber* L.) was assessed on MCF-7 cells. It induced apoptosis on the cancer cells, with corressponding suppression of anti-apoptotic proteins Bcl-2 and Bcl-xl [114].

2. **Colorectal Cancer**: Lupeol decreased cell viability in DLD1 and HCT116 cells and induced apoptosis, specifically in cells exhibiting a constituitively active Wnt signalling pathway. Treatment with lupeol was concurrent with decreased β-catenin transcriptional activity which resulted in decreased expression of Wnt pathway target genes of [115].
3. **Gall Bladder Cancer**: Lupeol inhibited cell growth, proliferation and induced apoptosis in GBC-SD cells; it also inhibited cell migration, cell invasion and reduced the expression of phosphorylated EGFR, phosphorylated Akt and MMP-9. In the same study, it was also observed that lupeol decreased the weight and volume of tumour in a GBC-SD xenograft model established in male BALB/c nude mice. In the *in vivo* system, a downregulation of phosphorylated EGFR, MMP-9 and PCNA were also observed [116].
4. **Liver Cancer**: Lupeol treatment inhibited the growth of MHCC-LM3, PLC-8024 and Huh-7 cells in a dose-dependent manner. T-ICs (tumour inititiating cells-cancer stem cells) of liver are thought to possess a significant chemoresitance to therapeutics; lupeol treatment inhibited the self-renewal capabilities of T-ICs of Huh-7 and PLC-8024 cells, and in the cells obtained from clinical samples. Lupeol also decreased the extent of tumourigenicity in Huh-7 and PLC-8024 cells *in vitro* as well as *in vivo* nude mice model, concurrent with decreased expression of CD133 (marker for liver T-ICs). It is probable that lupeol decreased chemoresistance by decreasing the expression of ABCG2, via regulation of the PTEN (phosphatase and tensin homolog; downregulation of PTEN is associated with self-renewal and chemoresistance)-Akt pathway [117]. Lupeol also inhibited cell viability in a dose-dependent manner and induced apoptosis in SMMC7721, concurrent with increased expression of caspase-3 and decreased expression of death receptor-3 (DR3) [118].
5. **Oral Cancer**: Lupeol induced growth inhibition and apoptosis on TU159 and MDA1986 cells. Reduced expression of NF-κB (a transcription factor required for cell survival) associated with decreased cell growth was observed for both lupeol alone and in combination treatment of lupeol and cisplatin; the effect was found to be weaker in TU159 cells. Combination treatment induced apoptosis possibly by cleaving caspase-3 [119].
6. **Pancreatic Cancer**: In AsPC-1 cells, lupeol inhibited growth and induced apoptosis; apoptotic induction corresponded with poly (ADP-ribose) polymerase cleavage, increased activity of Bax protein, increased expression of caspase-3, caspase-8 and caspase-9, activation of Erk1/2 and inhibition of phosphorylated p38. Lupeol also reduced the expression of Ras (malfunctioning Ras pathway is a common occurrence in cancer, and it leads to the accumulation of Ras protein), ODC(an oncogene), PKCα and NFκB [120].
7. **Prostate Cancer**: Androgen, required for normal prostate development, acts through androgen receptor; mutations of this receptor may contribute to generation of prostate cancer [121]. Lupeol preferentially inhibits the growth LAPC4 (androgen dependent expressing wild androgen receptor), LNCaP (androgen dependent expressing functional T877A mutant androgen receptor), 22Rv1 (androgen independent but functional, expressing functional H874Y mutated androgen receptor) and C4-2b (castration resistant phenotype, expressing functional H874Y mutated angrogen receptor) cells. Lupeol suppressed R1881 (androgen analogue) induced transcriptional activity of androgen receptor, and consequently the expression of target gene PSA (prostate specific antigen, a marker protein for prostate cancer diagnosis and prognosis). Lupeol also sensitized C4-2b cells to angrogen therapy; lupeol pre-treated cells when treated with bicalutamide (anti-androgen chemotherapeutic used for treatment of prostate cancer) showed greater extent of cell growth inhibition, than using only bicalutamide. Lupeol also inhibited *in vivo* tumourigenesis in nude athymic mice, who were implanted with C4-2b cells [122]. Lupeol also inhibited the cell proliferation of PC-3 cells, which was associated with G2/M arrest, suppression of cyclin B, cdc25C, plk1 and increased expression of 14-3-3σ genes. Induction of apoptosis in the PC-3 cells, with corresponding increased expression of bax, caspase-3, caspase-9, apaf1(apoptotic protease activating factor 1, a protein involved in apoptosis) genes and decreased expression of bcl-2 gene [123].
8. **Skin Cancer**: Apoptosis was induced in A431 cells by lupeol in a dose dependent manner. Apoptotic inducetion occurred via the intrinsic pathway corresponding with increased activity of Bax, caspases, Apaf1, suppression of Bcl-2 and cleavage of poly(ADP-ribose) polymerase. Lupeol also inhibited Akt/PKB pathway (an intracellular pathway involved in cell proliferation) and inhibited cell survival by suppressing NF-κB and increasing activity of IκBα (inhibitor of NF-κB) [124]. In an *in vivo* study carried out using CD-1 mice, TPA (12-O-tetradecanoylphorbol-13-acetate) was topically applied to mice skin to induce carcinogenic effects. Lupeol could inhibit TPA induce skin oedema, reduced epidermal hyperplasia (when mice skin was pre-treated with lupeol prior to TPA treatment). Lupeol also inhibited TPA induced epidermal ODC activity (in a dose-dependent manner) and epidermal COX-2 levels, and increased expression of induced nitric oxide synthase. Inhibition of PI3K/Akt pathway and NF-κB was also observed. NF-κB activity was suppressed by preventing phosphorylation of IκBα and suppressing the DNA- binding activity of NFκB [125].
9. **Tongue Cancer**: Lupeol induced growth inhibition and apoptosis on CAL27 cells. Treatment Treatment with lupeol alone and combination treatment with cisplatin reduced expression of NF-κB. Lupeol alone could prevent cell invasion by reversing the NF-κB dependent epithelial to mesenchymal transition. Lupeol also decreased the tumour volume in an orthotopic implantation of CAL27 cells in nude mice model and induced cell death by apoptosis and necrosis [119].

3.2.4 Betulin

The triterpenoid betulin is also known as betulinol, betuline, trochol, lup-20 (29)-ene-3b etc. It has a molecular weight of 442.728 g/mol and the molecular formula is $C_{30}H_{50}O_2$ [126]. Betulin is a pentacyclic lupine-type triterpenoid molecule. It has been isolated from many plants such as *Diospyros* leucomelas Poir., *Ziziphus mauritiana* Lam., *Nelumbo nucifera* Gaertn. etc. [127].

A list of cancers and cancer cell lines whose growth was inhibited by betulin has been given in table 4.

Table 4: List of different types of cancers and cancer cell lines whose growth and progression was inhibited by betulin

Organ affected	Name of cancer	Cell line affected	Reference(s)
Blood	Chronic Myelogenous Leukemia (CML)	K562	[128]
	Chronic Myelogenous Leukemia (CML)	K562-Tax (paclitaxel resistant)	[129]
	Acute Promyelocytic Leukemia (AML)	HL-60	[127, 130]
	Histiocytic Lymphoma	U937	[127, 130]
	Acute T Cell Leukemia	Jurkat E6.1	[127, 131]
Breast	Breast adenocarcinoma	MCF-7	[127, 128]
	Ductal carcinoma	T47D	[127, 131]
Cervical	Cervical adenocarcinoma	HeLa	[128, 132]
Colorectal	Colorectal adenocarcinoma	HT-29	[127, 131]
	Dukes' Type C, Colorectal adenocarcinoma	DLD-1	[127, 133]
	Colon carcinoma	Col2	[134]
Gastric	Gastric adenocarcinoma	EPG85-257P	[135]
	Gastric adenocarcinoma	EPG85-257RNOV (atypical mitoxantrone MDR variant)	[135]
	Gastric adenocarcinoma	EPG85-257RDB (classical daunorubicin variant)	[135]
Liver	Hepatocellular carcinoma	HepG2	[128, 136]
	Hepatocellular adenocarcinoma	SK-HEP-1	[128]
	Hepatocellular carcinoma	Hep3B	[136]
Lung	Lung adenocarcinoma	Lu1	[134]
	Large cell lung carcinoma	NCI-H460	[128]
	Lung carcinoma	A549	[127, 128, 131, 132, 133]
Neuroblatsoma	Neuroblastoma	GOTO	[127, 130]
	Neuroblastoma	NB-1	[127, 130]
	Neuroblastoma	SK-N-AS	[127, 131]
Ovarian	Ovarian endometrioid adenocarcinoma	A2780	[127, 137]
Pancreatic	Pancreatic adenocarcinoma	EPP85-181P	[135]
	Pancreatic adenocarcinoma	EPP85-181RNOV (atypical mitoxantrone MDR variant)	[135]
	Pancreatic adenocarcinoma	EPP85-181RDB (classical daunorubicin MDR variant)	[135]
Prostate	Grade IV, prostate adenocarcinoma	PC-3	[127, 128, 133]
	Prostate carcinoma	LNCaP	[134]
Skin	Epidermoid carcinoma	A431	[138]
	Malignant melanoma	G361	[128]
	Malignant melanoma	SK-MEL-28	[128]
Thyroid	Thyroid gland follicular carcinoma	FTC238	[131]

1. **Blood Cancer**: Betulin exhibited a weak inhibitory effect on cell proliferation of K562 cells [128]. It could also inhibit growth of paclitaxel-resistant K562-Tax cell lines [129] indicating that betulin has some potential to overcome drug resistance. According to Hata at al., 2003, betulin also inhibited growth in HL-60 and U937 cells [127, 130]. Antiproliferative effect of betulin was also observed in case of Jurkat E6.1, where betulin suppressed cell motility, altered cell morphology and induced apoptosis [127, 131]. In an another study, it was seen that combination of betulin and cholesterol induced apoptosis via the intrinsic pathway (corresponding with release of mitochondrial cytochrome c) in Jurkat cells. The number of dead cells were considerably greater in case of betulin-chlolesterol combination treatment, than compared to betulin alone. Although smaller concentrations of betulinic acid (a derivative of betulin, well known for its anti-cancer potential) can kill a larger number of cells compared to moderate concentrations of betulin, betulin only requires 24 hours to affect the cells, while in comparison betulinic acid requires 48-72 hours [132].
2. **Breast Cancer**: Betulin inhibited cell proliferation in MCF-7 cells [127, 128] and T47D [131]. In T47D, betulin inhibited cell motility, altered cell morphology and induced apoptosis [127, 131].
3. **Cervical Cancer**: Betulin suppressed proliferation in HeLa cells. This triterepene compound induced apoptosis in HeLa cells via the intrinsic pathway and apoptotic induction corresponded to release of mitochondrial cytochrome c, activation of caspase-9, caspase-3, caspase-7 and cleavage of poly (ADP)-ribose [128]. In the HeLa cells, combination treatment of cholesterol and betulin could induce apoptosis in a larger number of cells than compared to betulin treatment alone [132].
4. **Colorectal Cancer**: Betulin induced inhibition of cell proliferation to a noticeable extent in HT-29 cells. It also altered cell morphology, induced apoptosis and decreased cell motility [127, 131]. Betulin induced inhibition of cell proliferation was also observed in DLD-1 [127, 133] and Col2 cells [134].
5. **Gastric Cancer**: Betulin inhibited cell proliferation of EPG85-257P and drug resistant EPG85-257RDB (classical daunorubicin variant; daunorubicin is a cancer chemotherapeutic) and EPG85-257RNOV (atypical mitoxantrone MDR variant; mitoxantrone is an antineoplastic agent) cells. The IC_{50} value for betulin was found to be noticeable higher than that of betulinic acid [135].
6. **Liver Cancer**: Betulin inhibited cell proliferation in HepG2 and SK-HEP-1 cells [128]. This triterpenoid induced late Go/G1 phase and G2/M cycle arrest in HepG2 and Hep3B cells, indicating differential effects of

betulin on different liver cancer cell lines [136].

7. **Lung cancer**: Betulin decreased cell proliferation in A549 [127, 128, 131, 133], NCI-H460 [128] and Lu1 cells [134]. In another study, betulin altered morphological characters and induced apoptosis in A549 cells [131]. Combination treatment of cholesterol and betulin induced apoptosis in a larger population of A549 cells than that induced by betulin alone [132].
8. **Neuroblastoma**: Hata *et al.*, 2003 noted betulin induced inhibition of cell proliferation in GOTO, NB-1 [127, 130] and to noticeable extent in SK-N-AS [131]. In SK-N-AS, betulin suppressed cell migration, altered characteristics of cells and induced apoptosis [127, 131].
9. **Ovarian Cancer**: Chaturvedula *et al.*, 2003 [137] reported the inhibitory effect of betulin on the cell proliferation of A2780 cells [127].
10. **Pancreatic Cancer**: Betulin inhibited cell proliferation in EPP85-181P and drug-resistant varities EPP85-181RNOV (atypical mitoxantrone MDR variant) and EPP85-181RDB (classical daunorubicin MDR variant). Birch bark extract (which contains about 91% betulin and 4% betulinic acid) was found to be more effective for 257P cells [135].
11. **Prostate Cancer**: Betulin inhibited cell proliferation in PC-3 cells [127, 128, 133] and LNCaP cells [134].
12. **Skin Cancer**: Betulin induced inhibition of cell proliferation was seen in case of A431 cells [138] and according to Hata *et al.*, 2003 [131] in G361 and SK-MEL-28 cells as well [128].
13. **Thyroid Cancer**: Betulin, at concentrations of 5μM and 10μM, induced a dose-dependent shrinkage of FTC238 cells; the cells appeared to have an elongated appearance [131].

3.2.5 β-sitosterol

Chemically β-sitosterol is also known as 24-ethylcholest-5-en-3 beta-ol, 3beta-stigma-5-en-3-ol, 3beta-sitosterol, clionasterol, Harzol etc. The molecular weight is 414.718 g/mol and the molecular formula is $C_{29}H_{50}O$ [139]. β-sitosterol is widely distributed in the plant kingdom and is found in plants such as *Salvia macrosiphon* Boiss. [140], *Tephrosia purpurea* (L.) Pers. [141], *Coccinia grandis* (L.) Voigt [142], *Solanum xanthocarpum* Schrad & H. Wendl. [143], *Vitis vinifera* L. [144] *etc.* Sayeed *et al* have hailed β-sitosterol as "a promising but orphan neutraceutical to fight cancer". In recent years β-sitosterol has indeed shown great promise as a potential anti-cancer agent, specially for breast, prostate, colon, lung, stomach, ovarian and leuekemic cancers [145].

A list of cancers and cancer cell lines whose growth was inhibited by lupeol has been given in table 5.

Table 5: List of different types of cancers and cancer cell lines whose growth and progression was inhibited by β-sitosterol

Organ affected	Name of cancer	Cell line affected	Reference(s)
Blood	Histiocytic leukemia	U937	[146]
Breast	Breast adenocarcinoma	MCF7	[147, 148]
	Breast adenocarcinoma	MDA-MB-231	[147, 148, 150]
Cervical	Cervical squamous cell carcinoma	SiHa	[151]
Colorectal	Colorectal adenocarcinoma	HT-29	[152, 153]
	Colon adenocarcinoma	COLO 320DM	[154]
Gastric	Gastric adenocarcinoma	SGC-7901	[157]
Lung	Rat hepatoma	A549	[158]
Prostate	Prostate carcinoma	LNCaP	[159]
	Grade IV, prostate adenocarcinoma	PC-3	[160]
Skin	Epidermoid carcinoma	A431	[158]

1. **Blood cancer**: β-sitosterol induced growth inhibition and apoptosis in U937 cells. Apoptotic induction corresponded to decreased expression of Bcl-2 with increase in Bax/Bcl-2 ratio, degradation of poly-(ADP-ribose) polymerase, DNA degradation, decreased activity of phospholipase c (PLC)-gamma 1 protein (PLC is an important component of many signalling pathways and may be involved in tumourigenesis) and activation of caspase 3 [146].
2. **Breast cancer**: β-sitosterol inhibited MCF-7 and MDA-MB-231 cell growth, and induced apoptosis by activating Fas signalling (corresponding to increased Fas levels and caspase-8 activities) [147]. In a different study, it was determined that combination treatment of β-sitosterol and anti-oestrogenic chemotherapeutic drug tamoxifen inhibited growth in both MCF-7 and MDA-MB-231 cell lines, whereas tamoxifen treatment alone did not inhibit cell proliferation in MDA-MB-231. Ceramide levels were found to be increased by individual treatment of β-sitosterol and tamoxifen, and it was found to be even higher under the effect of combination treatment [148]. Ceramide, generated by hydrolysis of plasma membrane phospholipid sphingomyelin, acts as a second messenger in the apoptotic signalling cascade [149]. β-sitosterol increased ceramide synthesis probably by stimulating the enzyme palmitoyltransferase activity [148]. The signalling molecule TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) is known for apoptotic induction in many cancerous cells, while normal cells remain unaffected in both *in vivo* and *in vitro* systems. However, MDA-MB-231 cells are TRAIL resistant. Combination treatment of β-sitosterol and TRAIL induced apoptosis in these cells, which corresponded to increased activity of caspase-3, caspase-8 and caspase-9 and decreased expression of anti-apoptotic protein Bax [150].
3. **Cervical Cancer**: β-sitosterol inhibited cell proliferation of SiHa cells and induced mitotic arrest by inhibiting polymerization of microtubules. Decreased expression of both tubulin-α and MAP2 (microtubule associated protein 2) was observed [151].
4. **Colorectal Cancer**: β-sitosterol induced inhibition of cell proliferation in HT-29 cells [152, 153]. β-sitosterol, isolated from *Asclepias curassavica* L. inhibited growth in COLO 320DM cells [154]. The activity of PCNA (proliferating cell nuclear antigen- a doughnut-shaped protein that facilitates DNA replication and maybe partly responsible for driving tumour formation [155]) and β-catenin (a transcription factor that maybe mutated in many colon cancer cell lines [156]) are also suppressed [154]. β-sitosterol supplementation when supplied to reduced the number of rats affected with 1,2-Dimethylhydrazine induced colon

carcinogenesis and also decreased the number and extent of formation of aberrant crypt foci [154].

5. **Gastric Cancer**: β-sitosterol induced apoptosis in SGC-7901 cells; increased activity of pro-caspase-3 and Bax, and downregulation of Bcl-2 protein were observed [157].
6. **Lung cancer**: β-sitosterol induced a relatively minor but noticeable inhibition of cell proliferation in A549 cells [158].
7. **Prostate Cancer**: In LNCaP cells, the number of cells where β-sitosterol induced apoptotic cell death was considerably higher in comparison to cholesterol (a common animal sterol). Apoptosis was accompanied by an increase of 50% of ceramide production, indicating that apoptosis induction occurred via the sphingomyelin cycle even in LNCaP cells [159]. β-sitosterol supplementation inhibited PC-3 cell growth to a gretear extent than resveratrol (a phytosterol well known for its antioxidative efficacies), while combination treatment of both phytosterols induced cell death in even greater number of cells. β-sitosterol increased ROS production as opposed to resveratrol which decreased ROS production [160].
8. **Skin Cancer**: β-sitosterol had little to no effect on the cell growth and cell death of A431 cells [158].

3.2.6 Stigmasterol

The compound stigmasterol, with a molecular formula of $C_{29}H_{40}O$, has a molecular weight of 412.702 g/mol. The compound is also chemically known as Stigmasterin, Beta-Stigmasterol etc etc. [161].

Stigmasterol isolated from the marine alga *Navicula incerta* have been shown to induce apoptosis in HepG2 (hepatocarcinoma) cells, probably via the intrinsic pathway. Apoptosis induction was accompanied by increased expression of pro-apoptotic proteins Bax and p53, and decreased activity of the anti-apoptotic protein Bcl-2 [162]. In another study, 99.9 pure stigmasterol was isolated from *Azadirachta indica* A. Juss. The isolated stigmasterol showed chemopreventive activity in 7,12-dimethylbenz[a]anthracene (DMBA)-induced skin cancer of Swiss albino mice. Oral administration of stigmasterol resulted in reduction of both tumor size and total number of papillomas. A significant decrease in the activity of serum enzymes, such as aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and bilirubin in the experimental animals. Increased activity of glutathione, superoxide dismutase and catalase was also obsereved, indicating that stigmasterol enacted antioxidative functions as well [163].

4. Conclusion

Undoubtedly, cancer is one of the most dangerous non-communicable disease of our times, occupying second position after cardiovascular disease in terms of worldwide mortality and morbidity. As mentioned before, the chemically synthesised drugs cause a number of side effects in patients undergoing chemotherapy for cancer treatment. It is to be noted, however, that the plant-derived anti-cancer drugs available commercially are also known to cause side effects in a few cases. Hence, development of new anti-tumour drugs is of utmost importance. Since the plant kingdom is known to possess a number of bio-active compounds, many of which are still undiscovered, it is presumed that some of these phyto-constituents may possess cytotoxic effects specifically against tumour cells and therefore can be used used to develop new anti-neoplastic drugs.

The fact that crude extracts of *H. auriculata* showcase anti-tumour activities is enough to merit an investigation regarding the anti-tumour efficacies of this plant. Among the different phytochemicals reported from this plant, six compounds are already noted for their anti-cancer potency. These compounds have inhibited the growth of tumour cells and induced cell death, mostly by apoptosis, and in a few cases by necrosis. However, anti-cancer research using these phytoconstituents specifically isolated from this plant has not been reported yet. It is possible, that one or more compounds are yet to be discovered that may contribute to the plant's anti-tumour efficacies.

The number of reported cancer cases will increase exponentially in the coming years. Hence, all possible avenues of anti-cancer drug development must be investigated. It can be conclusively said that *H. auriculata* has some potential to be a source of new anti-cancer drugs. A thorough investigation regarding the anti-neoplastic potential of *H. auriculata* is required and the phytoconstituent(s) responsible for the plant's anti-tumour potency must be determined.

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