Review Article

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ANTICANCER POTENTIAL OF SOME AYURVEDIC PLANTS OF NORTH EASTERN INDIA: A COMPREHENSIVE PHARMACOLOGICAL REVIEW

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ABSTRACT

The paper highlights some of the most important Ayurvedic medicinal plants of North Eastern India having anticancer potential. A brief review of distribution and pharmacological study (both in vivo and in vitro) of ten Ayurvedic medicinal plants of the region published by various researchers is illustrated in this paper. The medicinal plants discussed here are *Enhydra Fluctuans* Lour (Sanskrit: Hilamochika), *Ageratum conizoides* Linn. (Sanskrit: Visamustih), *Dillenia pentagyna* Roxb. (Sanskrit: Aksikiphala, Nagakesaram), *Potentilla fulgens* Wall. (Sanskrit: Bajradantii), *Taxus baccata* Linn. (Sanskrit name: Talispatra), *Mirabilis jalapa* Linn. (Sanskrit name: Krisnakeli), *Xanthium strumarium* Linn. (Sanskrit name: Sarpakshi), *Dillenia indica* Linn. (Dilleniaceae) (Sanskrit name: Avartaki), *Alstonia scholaris* R.Br. (Apocynaceae) (Sanskrit name: Saptaparna) and *Azadirachta indica* A. Juss. (Meliaceae) (Sanskrit name: Nimba).

Key Words: Anticancer, Ayurvedic, North Eastern India

INTRODUCTION

Ayurveda, an ancient system of medicine originated in India described various formulations for the prevention and treatment of numerous diseases. Charaka and Sushrutta samhitas describe cancer as either Granthi (minor neoplasm) or Arbuda (major neoplasm). The basic principle of Ayurveda for the management of cancer is based on the idea of balance in bodily systems termed as Tridoshas (i.e. pitta or fire, vata or air and kapha or water) and uses mostly herbal treatment and dietary management[1].

India is a huge reservoir of medicinal plants. There are approximately 8000 species of medicinal plants found in India distributed in Western Ghat, North Eastern India and the Himalayan region[2]. North Eastern India comprised of Assam, Arunachal Pradesh, Nagaland, Meghalaya, Mizoram, Manipur, Tripura and Sikkim, covering an area about 225036 sq.km., situated between 22^0 19/ - 29^0 4/ North longitude and 89^0 42/ - 97^0 12/ East longitude and exists diverse type of medicinal plants[3]. Considerable works have been done on these plants to treat cancer, and some plant products have been marketed as anticancer drugs, based on the traditional uses and scientific reports. This review article describes ten Ayurvedic medicinal plants of North Eastern region of India, which are the natural sources of anticancer agents. A brief review of their distribution and pharmacological study published by various workers from the region is illustrated in this article.

DISCUSSIONS

The medicinal plants discussed here are *Enhydra Fluctuans* Lour (Sanskrit: Hilamochika), *Ageratum conizoides* Linn. (Sanskrit: Visamustih), *Dillenia pentagyna* Roxb. (Sanskrit: Aksikiphala, Nagakesaram), *Potentilla fulgens* Wall. (Sanskrit: Bajradantii), *Taxus baccata* Linn. (Sanskrit name: Talispatra), *Mirabilis jalapa* Linn. (Sanskrit name: Krisnakeli), *Xanthium strumarium* Linn. (Sanskrit name: Sarpakshi), *Dillenia indica* Linn. (Dilleniaceae) (Sanskrit name: Avartaki), *Alstonia scholaris* R.Br. (Apocynaceae) (Sanskrit name: Saptaparna) and *Azadirachta indica* A. Juss. (Meliaceae) (Sanskrit name: Nimba).

Enhydra Fluctuans Lour (Asteraceae)
(Sanskrit: Hilamochika)

Enhydra fluctuans is a hydrophytic plant and mostly found on wet roadside canals and marshy waste places between the months of November to January. It is highly prevalent in Bangladesh, Malaysia, China and the rest of South East Asia and Tropical Africa. In India, this plant is predominantly found in the North-Eastern region and mostly in Assam, Manipur, Meghalaya and Tripura[4].

Flavonoids obtained from *Enhydra fluctuans* (FEF) showed anticancer activity against Ehrlich's ascites carcinoma (EAC) bearing Swiss albino mice. The anticancer activity was assessed by measuring the tumor growth response, percentage increase of life span, hematological parameters, lipid peroxidation, and antioxidant enzyme activity, like GSH and CAT. Two flavonoids, baicalein 7-O-glucoside and baicalein 7-O-diglucoside, were isolated from the ethyl acetate fraction. Treatment with FEF caused a significant decrease in the tumor cell volume and increase of life span. All the hematological parameters, malonaldehyde content and antioxidant enzyme activity were restored towards the normal level. FEF was found to be cytotoxic in the in-vitro model[5].

Ageratum conizoides **Linn. (Asteraceae)**
(Sanskrit: Visamustih)

Aqueous extract of *Ageratum conizoides* roots at the dose of 100mg/kg/day showed antitumor activity against murine ascites Dalton's lymphoma. The extract increased the life span of tumor bearing mice by 27%[6].

Petroleum ether extract of *A. conyzoides* exhibited a significant inhibition on human gastric carcinoma (SGC-7901), human colon adenocarcinoma (HT-29), and mouse leukemia (P-388) cancer cell lines in-vitro. Ethylacetate extract of *A. conyzoides* showed a significant inhibition on man non-small cell lung carcinoma (A-549), SGC-7901, HT-29, P-388, human breast carcinoma (MDA-MB-231) and human breast carcinoma (DU-145) cancer cell lines. Furthermore, ethanol extract of *A. conyzoides* had a significant inhibitory activity on HT-29 and P-388 cancer cell lines. The ethanol extract showed an IC_{50} value of 1.73µg/ml in P-388 cell line, while petroleum ether extract had IC_{50} values of 14.06, 13.77, and 0.71µg/ml in A-549, SGC-7901, and P-388 cells, respectively. Similarly, the ethylacetate extract showed IC_{50} values of 0.68, 9.97, 14.88, and 0.0003µg/ml in A-549, DU-145, SGC-7901, and P-388 cells, respectively[7].

The cytotoxic effect of crude extracts as well as fractions of hydroethanolic leaf extracts of *A. conyzoides* was measured on leukemic (Jurkat), prostate cancer LNCap), breast cancer (MCF-7) and normal human prostate (PNT2) cell lines. The leaf, flower and whole plant extracts were slightly cytotoxic ($p<0.0001$) at the concentration range, 0-1000µg/ml compared to curcumin, 0-100µg/ml. In particular, the leaf extract exhibited the highest cytotoxicities in all three cancer cell lines. Among the cell lines, Jurkat cells were most susceptible to cytotoxicity of the leaf extract (15.1±0.3µg/ml) followed by LNCap (304.2±71.5µg/ml) and MCF-7 (934.9±105.9µg/ml) cells, but this effect was lower as observed for the remaining extracts. The petroleum ether, chloroform and ethylacetate fractions of the crude leaf extract were also slightly cytotoxic compared to curcumin within the concentration range, 0-100µg/ml ($p<0.0001$), with the chloroform and ethylacetate fractions exhibiting the highest cytotoxicities on all three cancer cell lines. Among the cell lines, the Jurkat cells (6.4±1.6µg/ml; 4.6±0.1µg/ml) were most susceptible to the cytotoxic effects of chloroform and ethylacetate fractions, followed by LNCap (35.3±6.5µg/ml; 37.3±2.1µg/ml) and MCF-7 (74.3±8.2µg/ml; 67.38±1.7µg/ml). However, this effect was lesser for the remaining fractions. The plant extracts as well as fractions were further tested on human normal prostate cell line (PNT2 to determine their selectivity index (SI) values. The ethanolic leaf extract exhibited the highest selectivity on Jurkat (SI=66.3) more than other cancer cell lines. This selectivity was greater than that found in the positive control, curcumin (SI=2.3). Also, the cytotoxic selectivities of chloroform and ethylacetate fractions were 7- and 9-fold, respectively compared to curcumin on Jurkat cells[8]. In Brine shrimp lethality bioassay, the cytotoxicity exhibited by crude methanolic extract of *A. conyzoides* stem was found promising with LC_{50} value 1.32µg/ml, comparing with the LC_{50} (0.689µg/ml) values of vincristin sulphate[9]. Treatment of Human lung cancer cell lines (SK-LU 1 and SK-MES 1) and human skin fibroblast cell line (FS5 cells) with ethanol extract of *A. conyzoides* leaves, and its petroleum ether, chloroform and ethyl acetate fractions at various concentrations (3.9µg/ml-2mg/ml) for 24h showed that SK-MES 1 cells were more susceptible to treatment with the plant fractions[10].

Dillenia pentagyna **Roxb. (Dilleniaceae),**
(Sanskrit: Aksikiphala, Nagakesaram)

Methanol extract of *Dillenia pentagyna* stem bark dose dependently increased the survivability of tumor bearing mice. Among different doses used 20mg/kg/day showed comparatively better antitumor activity (% ILS ~ 75%) against ascites Dalton's lymphoma[6]. Ethanol extract of *Dillenia pentagyna* stem bark showed potent antitumor activity, i.e. % ILS ~ 55% and % ILS ~ 48% at a dose of 50 and 100mg/kg/day, respectively, in murine ascites Dalton's lymphoma model. Chloroform and n-butanol fraction extracts of *D. pentagyna* also exhibited antitumor activity. Chloroform fraction extract of *D. pentagyna* showed highest antitumor activity (% ILS ~ 89%) at a dose of 50mg/kg/day[11].

Potentilla fulgens **Wall. (Rosaceae),**
(Sanskrit: Bajradantii)

Aqueous extract of *Potentilla fulgens* root showed potent antitumor activity, i.e. % ILS ~ 37% and % ILS ~ 36% at a dose of 50 and 100mg/kg/day, respectively in murine ascites Dalton's lymphoma model[11].

Methanolic (MeOH), butanolic (BuOH) and dichloromethane-MeOH extracts of *P. fulgens* L. roots showed *in-vitro* cytotoxic activity against various human cancer cell lines viz. leukemia (THP-1), liver (HEP-2), ovary (OVCAR-5) , lung (A-549), prostrate (PC-3) and neuroblastoma (SF-295). MeOH extract was found to be most potent and BuOH extract the least cytotoxic activity. While the MeOH extract exhibited maximum cytotoxicity against HEP-2 cancer cell line, DCM-MeOH extract showed significant activity against A-549 cancer cell line. BuOH extract showed no cytotoxic effect with the other cancer cell lines used for the study. The MeOH extract showed cytotoxic effects at higher concentrations (50µg/mL & 100µg/ml) against OVCAR-5, A-549, PC-3 and SF-295 cancer celllines, while DCM-MeOH extract at 100µg/ml exhibited significant cytotoxic effect against HEP-2 cancer cell line[12]. Methanolic (MeOH) extract of *P. fulgens* L. roots showed dose-dependent antitumor activity in Dalton's lymphoma cell model. Mean survival time is highest when the extracts were treated at the dose of 250mg/kg on 1st, 3rd, 5th and 7th days after transplantation of Dalton's lymphoma cells into the animals[13].

Taxus baccata **Linn. (Taxaceae)**
(Sanskrit: Talispatra)

Aqueous extract from *Taxus baccata* inhibited adenosine deaminase (ADA) activities in both cancerous and non-cancerous human gastric and colon tissues significantly. The ADA activities in human cancerous gastric and colon tissues were inhibited by 79.5 and 91.4%, respectively, in the study[14]. Aqueous and ethanolic extract of *Taxus baccata* leaf and Indian cow urine distillate showed synergistic anticancer effects as evidenced from good cytotoxicity and good recovery in clinico pathological parameters of Diethyl Nitrosamine induced hepatic cancer in mice[15].

Methanolic extracts of *T. baccata* leaves and seed cones was analyzed on human colon cancer cell line (HCT-116) and human breast cancer cell line (MDA-MB-231), using an MTT viability assay. The effect of both extracts was expressed by IC_{50} (inhibitory dose that inhibits cells growth for 50%). *T. baccata* leaf extract was cytotoxic on HCT-116 cells with IC_{50} values lower than 30µg/ml, which was considered good cytotoxic activity, while the extract of seed cones showed weaker effects.

The extracts did not produce significant cytotoxic effects on the MDA-MB-231 cell line[16]. Extract obtained from the bark of Iranian *T. baccata* showed a comparable cytotoxic effect to doxorubicin against Hela (Human cervix carcinoma) cells using MTT assay[17].

Taxane diterpneoid 2-deacetoxytaxinine J (2-DAT-J) isolated from the bark of Himalayan yew, *Taxus baccata* L. showed anticancer activity against breast cancer cell lines (MCF-7 and MDA-MB-231). 2-DAT-J showed significant *in-vitro* activity against breast cancer cell line at a concentration of 20µM and 10µM in MCF-7 and MDA-MB-231, respectively. 2-DAT-J was also tested for its *in-vivo* activity on DMBA-induced mammary tumors in virgin female Sprague Dawley rats at a dose of 10mg/kg body weight orally for 30 days and showed significant regression in mammary tumors as compared to vehicle treated group[18].

***Mirabilis jalapa* Linn. (Nyctaginaceae)**
(Sanskrit: Krisnakeli**)**

Petroleum ether extract of *Mirabilis jalapa* bark showed significant cytotoxic activity with the LC_{50} value 8.12µg/ml compared to vincristine sulphate (LC_{50} 0.33µg/ml) in brine shrimp lethality bioassay[19]. Two major proteins extracted from seeds of *Mirabilis jalapa* (27kDa and 62.5kDa in size) showed cytotoxicity on brine shrimp (LD_{50} of 95.50-489.78µg/ml at 24 and 48 hours). In addition, these proteins showed potent anticancer activity on Vero cells[20.] Ribosome-inactivating protein (RIP), a protein fraction isolated from the leaves of *Mirabilis jalapa* L was more cytotoxic to HeLa cell-line (LC50: 0.65mg/ml) than to Raji cell-line (1.815mg/ml) on 48 hours' incubation time[21]. MJ-30, a protein fraction (30kDP) with properties like ribosome-inactivating protein (RIP) isolated from the leaves of *Mirabilis jalapa* L. showed cytotoxic effect against T47D and SiHa cell line to different extent. The LC_{50} of the MJ-30 on T47D cell line and SiHa cell line were 0.36µg/ml and 5.6µg/ml, respectively. While in normal cells, represented by human mononuclear cells, MJ-30 was considerably less toxic, with LC_{50} of 21.04µg/ml. The study demonstrated that MJ-30 produced more cytotoxic activity toward breast and cervical cancer cells (58-fold and 4-fold, respectively) as compared to normal mononuclear cells[22]. Mirabilis antiviral protein (MAP) isolated from root extract of *Mirabilis jalapa* significantly inhibited HCT116, MCF7 and A549 cell lines with increasing of drug concentration. IC_{50} of MAP was observed to be 150µg/ml, 175µg/ml, 200µg/ml to HCT116, MCF-7, A549 respectively[23].

***Xanthium strumarium* Linn. (Asteraceae)**
(Sanskrit: Sarpakshi**)**

Active fractions of the methanolic extract of *Xanthium strumarium* showed potent cytotoxicity in microculture tetrazolium (MTT) and sulforhodamine B (SRB) assays in selected cancer cell lines *in-vitro*. The active fractions viz., chloroform soluble fraction of root, hexane soluble fraction of leaf, hexane soluble fraction of fruits and chloroform soluble fraction of fruits of *Xanthium strumarium* were tested in Dalton's ascitic lymphoma model in mice. The tumor bearing animals were treated with active fractions at two dose levels (100 and 200mg/kg). The extracts were found to increase the life-span of tumor bearing animals and restore the altered hematological and biochemical parameters significantly[24]. Hydro-alcoholic extract of *Xanthium strumarium* leaf at the concentration of 10mg/ml decreased the proliferation of L929 cancer cell lines[25].

***Dillenia indica* Linn. (Dilleniaceae)**
(Sanskrit: Avartaki)

Ethanolic and petroleum ether extracts of *Dillenia indica* stem barks showed cytotoxic effects in brine Shrimp lethality bioassay performed by observing mortality rate of brine shrimp nauplii (*Artemia salina*). The LC_{50} values observed by probity analysis were 574.926 and 334.284µg/ml for ethanolic and petroleum ether extracts, respectively[26].

Dillenia indica methanolic extract of bark and its n-hexane and ethyl acetate fractions possess potent cytotoxic principles with LC_{50} value17.68µg/ml, 17.68µmg/ml, 15.80µg/ml and LC_{90} value 486.61, 287.66, 148.82µg /ml, respectively, compared with positive control vincristine sulphate (LC_{50} 0.631 mg/ml and LC_{90} value 13.51mg/ml)[27].

Methanolic extract of *Dillenia indica* L. fruits showed significant anti-leukemic activity in human leukemic cell lines U937, HL60 and K562. Fractionation of the methanolic extract, on the basis of polarity showed that ethyl acetate fraction had highest anti-leukemic activity. A major compound, betulinic acid, was isolated from the ethyl acetate fraction by silica gel column chromatography and was identified and characterized. Betulinic acid could explain the anti-leukemic activity of the methanolic extract and the ethyl acetate fraction[28].

***Alstonia scholaris* R.Br. (**Apocynaceae)
(Sanskrit: Saptaparna**)**

Ehrlich ascites carcinoma-bearing mice injected with various doses of *Alstonia scholaris* extract (60-240mg/kg) in combination with berberine hydrochloride (8mg/kg) demonstrated that the combination of 180mg/kg of *Alstonia scholaris* extract with 8mg/kg of berberine hydrochloride exhibits greatest antitumor effect. Similarly, when 180mg/kg of the extract combined with different doses of berberine hydrochloride (2-12mg/kg), a dose-dependent increase in the anticancer activity was observed up to 8mg/kg of berberine hydrochloride. The efficacy of the combination of 180mg/kg of *Alstonia scholaris* extract was also tested with 6mg/kg body weight of berberine hydrochloride in various stages of tumorigenesis, and it was demonstrated to be effective when given in the early stages, although the efficiency decreased with an increase in the tumor developmental stages[29].

The anticancer effect of various doses of an alkaloid fraction of *Alstonia scholaris* (ASERS), was studied *in-vitro* in cultured human neoplastic cell lines (HeLa, HepG(2), HL60, KB and MCF-7). Treatment of HeLa cells with 25µg/mL ASERS resulted in a time dependent increase in the antineoplastic activity and the greatest activity was observed when the cells were exposed to ASERS for 24 h. However, exposure of cells to ASERS for 4 h resulted in 25% viable cells. Treatment of various cells with ASERS resulted in a concentration dependent decline in the viable cells and a nadir was reached at 200µg/ml in all the cell lines studied. The IC_{50} was found to be 5.53, 25, 11.16, 10 and 29.76µg/ml for HeLa, HePG2, HL60, KB and MCF-7 cells, respectively[30]. *Alstonia scholaris* bark extract showed chemopreventive potential in DMBA-induced skin tumorigenesis in Swiss albino mice[31].

Aqueous, hydro-alcoholic (50% ethanol) and ethanolic extracts of *Alstonia scholaris* (L.) R. Br. bark showed potent anticancer activity on Human Leukaemia Cell line HL – 60 *in-vitro* with GI_{50} values of 15.7, 13.0 and 13.4, respectively[32].

***Azadirachta indica* A. Juss.** (Meliaceae)
(Sanskrit: Nimba)

A crude aqueous extract from seeds and leaves of *Azadirachta indica* showed *in-vitro* antitumour and cytotoxic activity at different concentrations (250, 500, 750 and 1000µg/ml). The acidic extract from leaves and neutral extracts from the seeds inhibited Ehrlich ascites carcinoma cell line growth and had anticancer activity. Half maximal inhibitory concentration (IC_{50}) values of acidic extracts from leaves and neutral extracts from seeds were 669.43µg/ml and 724.63µg/ml, respectively[33].

CONCLUSION

It is clear from the study that North Eastern region of India is rich in precious Ayurvedic plants which can be used to treat cancer. Conventional cancer treatments using chemotherapy and radiation may be effective for treating cancerous tumors, but such treatments have potential toxic effects like improper functioning of the immune system, hair loss, skin rashes etc. But the application of Ayurvedic medicinal plants for cancer treatment has no such side effects and eco-friendly. Research in the field of ethnopharmacology would help in developing safe and effective anticancer drugs from these herbal resources. Botanical identification, authenication, quality production of herbal products, standardization, *in-vitro* and in-vivo pharmacological screenings of the medicinal plants, randomized controlled clinical trial are the important steps that should be taken in to consideration in the drug development programme. Since medicinal plants are the key source of active principle of Ayurvedic medicines, therefore, it is also becoming necessary to preserve the medicinal flora.

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