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A synoptic review on the anticancerous property of *Phyllanthus emblica* L. or *Emblica officinalis* Gaertn. (Indian gooseberry)

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Abstract
Medicinal plants have been long utilised in the history of mankind for their various pharmacological properties. One such novel medicinally important plant is *Phyllanthus emblica* L. or *Emblica officinalis* Gaertn., belonging to the family Phyllanthaceae. It is commonly known as Indian gooseberry or Amla, and has numerous references in the indigenous systems of traditional medicine, in the treatment of various ailments. Amla, which is rich in Vitamin C and other essential amino acids, along with a number of macro and micronutrients, is a plethora of several significant pharmacological properties. One of the remarkable properties of the plant is its anticancerous effect. Cancer being one of the most dreadful diseases worldwide and the scavenging effects of chemotherapeutic drugs on human bodies is a matter of deep concern. In this regard, the anticancerous property of the plant is of immense importance, as it can be utilised as an antineoplastic agent as well as a chemopreventive adjuvant drug. The plant owes this ability to the presence of active principles like flavonoids like quercetin, rutin, tannins, polyphenols like gallic acid, ellagic acid, progallin, etc. This review is an attempt to summarise the findings and reports of the effects of the various extracts of the plant on the different types of carcinomas.

Keywords: *Phyllanthus emblica* L., *Emblica officinalis*, amla, anticancerous, pharmacological property

Introduction
Phyllanthus emblica L. or *Emblica officinalis* is an arboreal plant belonging to the family Phyllanthaceae in order Malpighiales. It is widely distributed in tropical and sub-tropical nations like India, Bangladesh, Cambodia, Myanmar, Indonesia, Thailand, Vietnam, parts of China, etc. It is commonly known as Indian gooseberry or Amla and is mentioned in traditional systems of medicine like Unani and Ayurveda as one of the most potent therapeutic herbs. Amla or 'Amlaki' is considered as the best rejuvenative herb in both *Charak Samhita* and *Sushrut Samhita* of Ayurveda.

The novel plant is used in cure of numerous ailments like common cold, cough, fever, asthma, bronchitis, hypertension, diabetes, osteoporosis, skin allergies, oral disorders, and inflammatory diseases [1, 2]. It is also used as a laxative, diuretic, antipyretic and as a hair vitalizer. The plant possesses cardioprotective, neuroprotective, hepatoprotective, antihypercholesterolemic, antianemic, antidiarrheal, gastroprotective, anticarcinogenic and immunomodulatory properties [3, 4, 5, 6]. The anticancerous ability is reported to be due to its free radical scavenging, radiomodulatory and chemomodulatory properties [7]. The fruit extract of this plant is often being used as a nutrient drink possessing a rich content of vitamin C and essential amino acids.

P. emblica contains a number of nutritional components like polysaccharides, vitamins, minerals, amino acids; and many functional constituents like phenolic acids, tannins, flavonoids, sterols, triterpenoids, lignans, alkaloids, alkanes, aromatic micromolecules [8]. The fruit is a rich source of phosphorous, potassium, calcium, sodium, iron, sulphur, magnesium, selenium among other macro and micronutrients. The fruit extract has been reported to contain thrice the content of protein and 160 times the ascorbic acid as present in apples, as well as high concentrations of other essential amino acids such as glutamic acid, aspartic acid, alanine and lysine [9]. Iron is absorbed efficiently in presence of ascorbic acid and since amla is rich in both of these, it becomes an extremely potent source of vitamin C. In a 100 gms portion of *P. emblica* fruit, the vitamin C content ranges from 600 to 1300 mg [10]. Many compounds like

gallic acid, ellagic acid, 3-ethylgallic acid (3-ethoxy-4,5-dihydroxy-benzoic acid), chebulinic acid, quercetin, corilagin, isostrictiniin, etc were isolated and identified in the plant [11]. Hydrolysable tannins - emblicanin A and emblicanin B, are reported to possess antioxidative affects [12]. It also contains other tannins, such as punigluconin and pedunclagin.

Cancer is one of the most leading health problems worldwide. Various conventional treatment procedures including chemotherapy, radiotherapy, surgery are being followed along with new approaches of stem cell therapy, chemodynamic therapy, sonodynamic therapy, nanoparticle-assisted therapies are also being encouraged to cure carcinogenic ailments [13]. These techniques have a number of side effects with major physiological implications, affecting the patients negatively. Therefore, scientists have been trying to establish a natural alternative therapy utilizing phytochemicals derived from the numerous medicinally useful plants. Treatment with medicinal plant extract has been prevalent from time immemorial in the history of mankind and have evident references in the traditional systems of medicine. Naturopathy in the treatment of cancers is now being encouraged to reduce the ill-effects of synthetic chemotherapeutic drugs and radiations, and therefore, in this regard, the potency of the anticancerous property of *P. emblica* has to be evaluated more intensely.

Anticancerous activity of *Phyllanthus emblica*

Several evidences of the positive effects of *P. emblica* on the different types of cancers are reported and these data establish a strong framework of the plant to be utilised as a novel natural remedy towards combating the disease.

P. emblica fruit extract was administered orally for 7 days to mice, before they were exposed to lead and aluminium salts to examine the clastogenecity induced on the bone marrow chromosomes. It was reported that the fruit extract treatment reduced the chromosomal breakage significantly and increased the cell division frequency. It was compared with the effect of synthetic ascorbic acid and found to be more effective than the similar doses of the acid alone [14].

Swiss-albino mice were fed with the fruit extract for a continuous period of 28 days. They were then treated with the carcinogen - 3,4- benzo(a)pyrene from the 8th day on every alternative day for eight doses in total. Cytotoxic effects of the carcinogen were tested and it was found that the chromosomal aberrations and damaged cells were in a much-reduced number in the mice that were fed with the *P. emblica* extract than the control mice [15].

In an investigation, tumour bearing mice were treated with 1.25 g per Kg body weight of the aqueous extract of *P. emblica* and showed an increased life span of 20%. At a concentration of 16.5 µg/ml of the extract, the inhibition could be about 50%. The extract was found to have a significant effect in reducing the solid tumours [16].

Ethanolic extract of the fruit of *P. emblica* was orally administered to Swiss albino mice for seven consecutive days in doses of 100, 250, 500 mg/Kg of body weight. The mice were subjected to the carcinogen - 7,12-dimethylbenz(a)anthracene (DMBA). The fruit extract was reported to positively increase the liver antioxidants like glutathione, glutathione peroxidase, glutathione reductase and detoxifying enzyme glutathione-S-transferase in the mice and reduced the activating enzymes cytochrome (Cyt) P_{450} and Cyt b_5 in the liver cells, thereby exhibiting a hepatoprotective mechanism against the carcinogenic effect. This efficacy of the extract was found to be at the maximum at 500 mg/Kg concentration [17].

Similarly, Sprague-Dawley rats subjected with a dose of 0.25 mg/Kg of body weight of 7,12-dimethylbenz(a)anthracene (DMBA) to induce breast cancer were administered the fruit juice of *P. emblica*, which resulted in a decrease of lipid peroxidation markers, lipid profile and increased the antioxidant levels in the liver, plasma and mammary tissues. The reversal of proliferative effect due to the fruit juice treatment was also affirmed by histopathological and immunohistochemical analysis [18].

Chemopreventive efficiency of *P. emblica* fruit extract in reducing the number, incidence and yield of tumours was reported in 7, 12-dimethyabenz(a)anthracene (DMBA)-induced skin tumorigenesis in Swiss albino mice [19].

Wistar rats were subjected to diethylnitrosoamine (DEN) at a dose of 200 mg/Kg of body weight, followed by treatment of 2-acetylaminoflourine for a continuous period of 14 days. The rats that were pretreated with defatted methanolic extract of the amla fruit at doses of 100 and 200 mg/Kg of body weight, reported to exhibit partial recovery of pathological changes and also suppressed the tumour formation [20].

In another investigation, the efficiency of the fruit extract on the inhibition of cell growth of human cancer cell lines. It was reported that this extract at a concentration of 50-100 µg/mL could significantly inhibit the growth of cancer cells in specific lung, liver, cervical, breast, ovary and colorectal cancer cell lines. In HeLa (Cervical) cell line, the fruit extract could cause fragmentation of DNA and also increased the caspase 3/7 and 8 activities, along with upregulating Fas protein, thereby giving an indication of death receptor-mediated apoptosis pathway. The extract was also reported to have reduced about 50% of tumours, both in number and volume, when treated on mouse skin [21].

A study was conducted in cervical cancer cell lines - HPV16 and HPV18 positive- to examine the effect of *P. emblica* extract. The extract was reported to induce the down regulation of the AP-1 proteins, along with the suppression of the transcription of the virus, thereby inhibiting the growth of the cervical cancer cells. It was therefore suggested that the anticancer property of the plant extract is achieved by the inhibition of AP-1 and directly targeting the transcription of the viral oncogenes [22].

Phenolic compounds derived from fruits of *P. emblica* were assayed for their immunomodulatory and anticancerous property on the proliferative action of splenocyte and cytotoxic effect on human breast cancer cell and embryonic lung fibroblast cells. All the compounds - geraniin, quercetin 3-β-d-glucopyranoside, kaempferol 3-β-d-glucopyranoside, isocorilagin, quercetin, kaempferol and rutin exhibited cytotoxicity against the carcinoma cells at different concentrations. Geraniin and isocorilagin exhibited higher cytotoxicities than other compounds against the breast cancer cell line (MCF-7) [23].

The fruit extract of *P. emblica* was examined for its antiproliferative effect on ovarian cancer cells under *in vivo* and *in vitro* conditions. The fruit extract enhanced the expression of the autophagic proteins beclin1 and LC3B-II and reduced the expression of angiogenic genes like hypoxia-inducible factor 1α (HIF-1α) in OVCAR3 cells. Quercetin, a significant component of the fruit had similar effects in the *in vitro* conditions. Antiproliferative effects of the fruit was observed in mouse xenograft tumours also [24].

The polyphenol extract of leaves of *P. emblica* was subjected at different concentrations to cervical cancer (HeLa) cells. It was observed that the polyphenol extract could efficiently

inhibit the proliferation of cervical cancer cells at the concentration of 150 mg/ml. It induced the HeLa cell cycle arrest at G2/M phase and apoptosis was triggered. Activation of apoptotic marker proteins - Fas and FasL was induced, along with cleavage of caspase-8 [25].

Antimetastatic effect of aqueous extract of *P. emblica* was reported in human fibrosarcoma cells HT1080, where the extract pretreatment to the cells could down regulate the expression of matrix metalloproteinases - MMP2 and MMP9. This finding emphasized the use of the plant extract as a parallel adjuvant drug in chemotherapy [26].

P. emblica extract at a dose of 20-320 µg/ml increased the efficiency of mitomycin C and cisplatin, two widely used chemotherapeutic drugs. These drugs have been reported to have various genotoxic effects and genomic instability on the normal cells. The plant extract has been shown to efficiently reduced the mitotic index, blocking the mitotic progression and promoting apoptosis in these two drug-treated cells, in the study done in colorectal cancer. The genotoxicity caused by these drugs is significantly regressed by the plant extract treatment. It was reported in this study that the plant extract holds the potential of reducing the chances of secondary cancer formation induced by chemotherapeutic drugs [27].

Effect of *P. emblica* extract on hepatic cancer

Hepatocellular carcinoma is considered to one of the most common and fatal cancers worldwide [28]. Apoptosis is a significant physiological pathway in reduction of excess cell growth in case of inflammation and tumours. Tumour cells acquire apoptotic resistance by upregulating the expression of anti-apoptotic cells like Bcl-2 and repressing the expression of or mutating the proteins like Bax [29]. In this regard, several investigations have been done to study the effect of *P. emblica* on the ability to induce apoptotic pathways in hepatoma cell lines. Apoptosis was reported to be induced in the human hepatoma BEL-7404 cells, by the effect of Galic acid that is extracted from the leaves of *P. emblica*. Upregulation of expression of Bax and down regulation of Bcl-2 triggered caspase activation leading to apoptotic pathway [30, 31]. The effect of Progallin A was studied on human hepatocellular carcinoma BEL-7404 cells. Progallin A was isolated form the acetic ether part of leaves of *P. emblica*. Similar effects as Gallic acid were observed and reported. Progallin A induced apoptosis of the carcinoma cells that were related to G1/M and G2/M arrest and triggered the Bax expression while repressing the Bcl-2 gene expression [32].

Cholangiocarcinoma has a high degree of occurrence in northeastern part of Thailand. In a very recent study investigating the effects of extracts of *P. emblica*, *Terminalia chebula* and *Terminalia bellirica* on the cell lines of cholangiocarcinoma, it was reported that the highest efficiency in inhibition of inflammation and inducing apoptosis, was exhibited by *P. emblica* extract. This ability was attributed to the presence of highest content of flavonoids and optimum concentration of tannins [33].

Presently, studies are being undertaken to understand the molecular mechanism of Reactive Oxygen Species (ROS)-mediated induction of apoptosis in the human hepatic cancer cells [34].

A latest investigation was carried out to study the effect of *P. emblica* on carcinogens diethylnitosamine-induced hepatic and 1,2- dimethylhydrazine - induced carcinogenesis in rats. The rats were treated with the ethanolic extract of *P. emblica* fruits. It was reported that the extract reduced the size of the preneoplastic lesions and enhanced the liver antioxidant enzyme activities, further establishing the role of the plant in combating cancerous growth [35].

Radioprotective ability of *P. emblica*

Swiss albino mice who were pretreated orally with *P. emblica* extract at a dose of 100mg/Kg of body weight and were irradiated with gamma rays showed decreased level of lipid peroxidation and increase in the glutathione and catalase concentration in the intestine. This report established the radioprotective effect of the plant [36].

According to a recent finding, it was reported that tannins in *P. emblica* could trigger intercellular protein aggregation, leading to activation of interferons and eventually causing endoplasmic reticulum stress. This induced the immunogenic cell death in case of lung cancer cells. The tannins also enhanced the antitumour efficiency of cisplatin, a chemotherapeutic agent. This ability of *P. emblica* is attributed to the presence of gallocatechin, gallic acid, methyl gallate, ethyl gallate and ellagic acid, all of which could induce immunogenic death in *in vitro* conditions [37].

Summing up, the different mechanisms by which this novel plant extract exhibits its anticancerous property is by the free radical scavenging activity, by reducing cytochrome enzyme levels, anti-inflammatory activity to potentially reduce tumour incidence or reduce the size of the tumours [38]. The cell cycle regulation and controlling the oncogenic signalling genes are also implicated mechanisms of cancer inhibition [39].

Conclusion

Phyllanthus emblica L. is one of the most novel medicinally important plant that presents an array of pharmacological properties. Being the richest source of vitamin C and ascorbic acid, it is the most significant immunoprotective and immunostimulatory herb. The active principles present in the plant departs it the phenomenal ability to induce apoptosis in various carcinoma cells and thereby posing to be the most reliable natural alternative to inhibit cancerous growth and also reduce cytotoxicity to the normal cells during the conventional chemotherapeutic treatments. Antimetastatic effects on certain types of cancer cell lines have also been reported. The plant fruit extract also enhances the efficacy of certain widely used chemotherapeutic drugs. The number of reports and data presented in this review shows and establishes the phenomenal potential of the plant extract to reduce the number, yield, occurrence of tumours in many cancer lines like as well as increases the life expectancy of the animals having cancer, by a significant margin. Though, *P. emblica* is widely used as ayurvedic proprietary medicine, it should also be prevalently administered in the modern system of medical science as a natural therapeutic alternative. This review encompasses the latest findings of the plant's anticancerous property and encourages the possibility for more clinical trials.

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