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Photocatalytic degradation of synthetic food dye, sunset yellow FCF (FD & C yellow no. 6) by *Ailanthus excelsa* Roxb. possessing antioxidant and cytotoxic activity

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ABSTRACT

The purpose of our work is to identify the bioactive compounds of bark and leaves extract from *Ailanthus excelsa* Roxb. and to explore its effectiveness against synthetic food dye. The presence of primary and secondary metabolites was confirmed by carrying out phytochemicals analysis. With the prior knowledge accessible on the indispensable secondary metabolites holding antioxidant and cytotoxicity activity, the quantitative screening of total phenolic and flavonoid content in methanolic and aqueous extract of bark and leaves from *Ailanthus excelsa* were done. Comparatively, a higher value of flavonoid (161 ± 0.3 μg/mg) and phenolic acid content (152.4 ± 0.14 μg/mg) was found in bark extract. By FTIR analysis, the characteristic peak was obtained at 1581.63 and 1598.99 cm^{-1} confirmed the presence of functional groups associated to flavonoids and other phenolic groups respectively. In bark extract, 81% of DPPH inhibition was observed when compared to ascorbic acid (standard) 92% of free radical scavenging activity. Bark extract from *Ailanthus excelsa* exhibited 71% cytotoxicity against HeLa cell line (cervical cancer). In examining the toxicity level of crude extracts with red blood cells (RBC), the bark extract was showed a very less (2.8%) haemolytic activity. They also showed maximum zone of inhibition in antibacterial activity *i.e.* 13 ± 0.5 mm against *Escherichia coli* culture. At a concentration of 10 mg/mL of crude extract from *A. excelsa*, 55% degradation of sunset yellow dye was observed. It concludes that, the compounds present in the *A. excelsa*, especially the bark extract showed better photocatalytic, haemolytic, antioxidant, cytotoxicity and antibacterial activity when compared to leaves extract.

1. Introduction

From ancient period, in countries such as India, China, Egypt and Greece, terrestrial plants have been used to discover modern drugs [1]. In developing countries the traditional medicines having wide historical background are utilized for primary health care and in those plants only few have been explored for their potential as drug. India, a "Botanical garden of the World" has traditionally well practiced knowledge on herbal medicine. The World Health Organization has exhibited 2500 species with medicinal purpose in India and from that 150 species were used on larger scale [2].

Plants are the major source of natural secondary metabolites; which are besides being an essential requirement for the plants to adapt in the environment they also act as an active component by providing a range of potential health-beneficiary activities. The examination of natural source is desirable for development of novel bioactive chemo-types and molecular diversity of effective medicines [3]. By multidisciplinary approaches, plants can be involved alone or in combinatorial synthetic methodologies *i.e.* poly-herbal for their synergistic effects [4,5]. Screening of new natural compounds plays a vital role in identifying the plants which accomplish the following criteria lack in currently available drugs, i) counteract drug resistance, toxicity, side effects and specificity, ii) accomplishing chemical (presence of hydrophobic phenyl groups in compounds structure for easier cellular intake), pharmacological and toxicological studies [6,7] and (iii) with the input of new technologies, a re-establishment of plants and the high throughput screening of its potential are necessary as the molecules derived from plants are proving to be an efficacious source. The endogenous

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organism of plants acts against infection by conditioning the plants body tissues and by maintaining the body equilibrium. Plants can also be selected based on its ability to withstand the mutagens, stress caused by drought, desiccation and strong winds by adapting and altering its physiological and biochemical profile respectively [8].

Ailanthus excelsa is a large tree 18–25 m tall; trunk straight, 60–80 cm in diameter; produce slightly bitter aromatic and originated from China. It belongs to Simaroubaceae family and an indigenous plant to central and southern region of India. Leaves are alternate, large, pinnately compound, 30–60 cm and its leaflets are 8–14 pairs, long stalked, ovate or broadly lanced shaped, 6–10/90 cm length, 3–5 cm breadth, hairy gland, curved, long pointed; their edges are roughly toothed and lobed. It has high resemblance with the morphology of *Azadirachta indica* (neem tree) plant. It is an Egyptian traditional medicine and its light gray-brownish rough bark is used as appetizer, anthelmintic, febrifuge, astringent [9]; in dysentery, dyspepsia, ear ache, bronchitis, skin disease, rheumatism, rectum troubles, and it is also used to cure fever caused by tridosha, wounds, gout, skin eruptions and asthma [10]. In Indian folk medicine, it is widely used for fungal disorders [11] and gastroprotectivity. Leaves were reported to have hepatoprotective effect and antidiabetic activity [12].

Free radicals are released from various sources; such as aerobic metabolism, mitochondrial and respiratory leak, enzymatic reactions, auto-oxidant reactions, UV light and ionizing radiation. As carcinogen, they accumulate and involved in major part of cancer pathophysiology. They put forth their adverse effects in several ways like DNA damage, varying cell-signaling pathways and also manipulating gene expression; which are necessary during defense, inflammation, signal transduction, cell-cell adhesion, cell proliferation, transcription and apoptosis. Mortality threat of diseases can be shattered by intaking phytochemicals containing exogenous antioxidant that helps in inhibiting free radical generation and thereby making stronger antioxidant system in the body. In spite of its cancer preventing property, they can also hinder, interrupt or undo the carcinogenesis process from pioneer substance. It is well known that consuming flavonoids can reduce the occurrence and risk of cancer as mentioned in Fig. 1. Alkaloids such as canthin-6-one and 1, 5 & 8-methoxy canthin-6-one isolated from root bark of *Ailanthus altissima* have shown cytotoxicity against Epstein-Barr virus early antigen [13]. Quercetin from *A. excelsa* fruit was reported to acts against *Ehrlich ascites* tumor cells [14].

In standard herbal recommendation, terpenoids were used for its aromatic feature. A known compound apigenin and luteolin isolated from leaves extract of *Ailanthus excelsa* were inhibited the cell growth of A375 and C32 cell lines (amelanotic melanoma and malignant melanoma cells) [15]. Quassinoids is a highly oxygenated triterpenoid containing anti-cancer properties were previously reported in stem bark and aqueous extract of root from *Ailanthus excelsa*. It is also potent for anti-leukemic, antiviral, anti-malarial, anti-feedent, anti-asthmatic property. Other compounds like excelsin, ailanthinone, glaucarubol and polyandrol could fight against leukemia by inhibiting the protein synthesis of ribosomal peptidyl enzyme [16]. But some compounds are toxic causing epigastric pain, limbs tingling, myocarditis, substernal chest pressure in high dosage to be use clinically hence modifying the structure of the compounds to hold their activity with less toxicity are the proper way of drug usage.

AECHL-1 (*Ailanthus excelsa* extract-1) a triterpenoid, an isolates from root bark disturbs microtubule structure in MCF-7, the major requirement in mitosis, for suppressing p53 and cell proliferation. It inhibits cell growth by S/G2-M arrest in MDA-MB-231, MCF-7 (human breast adenocarcinoma) and in PC3 cells (human prostate cancer) whereas in B16F10 (*Mus musculus* skin melanoma) through p21 gene the cell cycle was arrested at G1 phase by inhibiting the CDK-cyclin kinase activity phase and complexing with CDK2, 4 and 6; increasing the expression of tumor suppressor proteins (p53/p21) and decreasing oncogene c-Myc expression (which is upregulated in cancer cells and helps to promote tumor growth) and thus down regulation of cyclin D1 and CDK4 phosphorylation of p53 at serine15 [17].

Additional conventional approaches accessible in cancer drug designing are deregulating ER stress, mitochondrial function and mitotic spindle of tumor cells. AECHL-1 elevates ER stress proteins such as PERK, IRE-1 and PKR and phosphorylates eIF2α (eukaryotic Initiation Factor 2). It also cause mitochondrial accumulation by releasing calcium as a secondary messenger from ER stores for mitochondrial mediated cell death in MCF-7, MDA-MB-231. Its activity is precise and competent in reducing tumor progression when compare to existing plant derived chemotherapeutic compound paclitaxel (diterpenoid) and

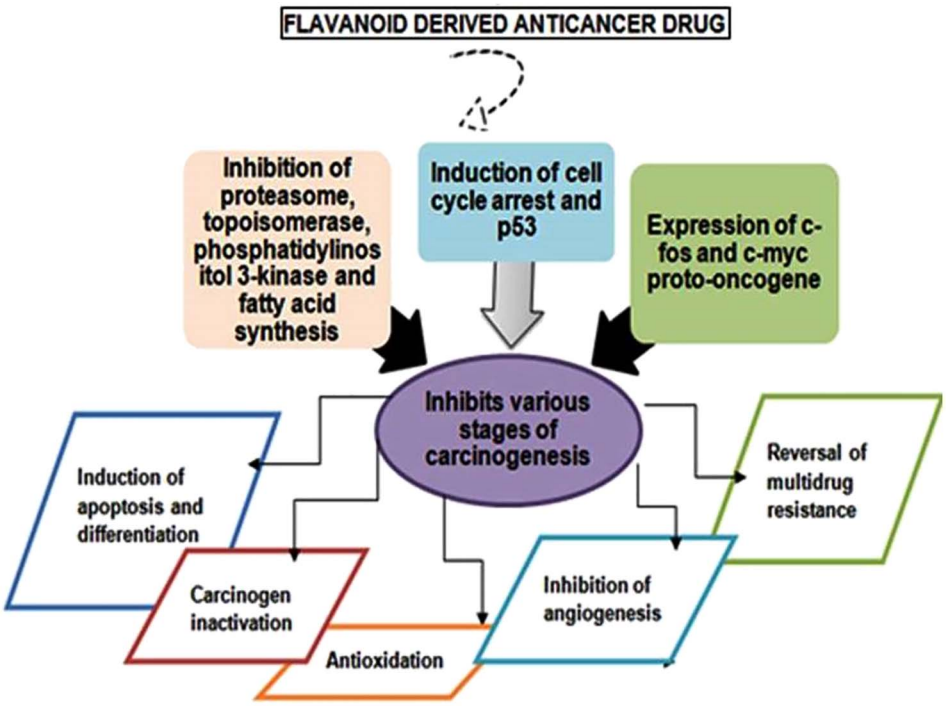

Fig. 1. Common anticancer mechanism of flavonoids [15].