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Title of the thesis: **Antiproliferative efficacies of various extracts from *Cassia fistula***

Cassia fistula (*family-Fabaceae*) has been used in Ayurvedic Medicine and also reported for various pharmacological properties. Due to the medicinal importance of this plant we hypothesized to investigate the antiproliferative efficacies.

C. fistula methanolic extracts of seed/pulp had high content of phenolic and flavanoid compounds, whereas hexane extracts of seed/pulp had more of terpenoid and steroid compounds. *C. fistula* pulp/seed extracts evaluated for antioxidant property (*in vitro*) by four widely used biochemical methods. Antioxidant property of the methanolic extracts of pulp/seed progressively increased with the increase of concentration, whereas, hexane extracts of pulp/seed showed antioxidant activity at very high concentration. Results depicted that antioxidant potential of these extracts were significantly correlated with the phenolics and flavanoids contents.

C. fistula methanolic extracts of pulp and seed showed antifungal activity with MIC values against *Candida albicans, Candida tropicalis* and *Candida glabrata* ranging between 100-250 and 300-350µg/ml respectively. However, MIC values of hexane extracts of pulp and seed ranged between 250-300 and 450-500µg/ml respectively. Hexane extracts of pulp/seeds showed ergosterol synthesis inhibition in the cell wall of candidal species followed by the methanolic extracts of pulp/seed. The results of ergosterol biosynthesis inhibition, MICs and growth patterns of *Candida* species suggested that the antifungal activity of hexane and methanolic extracts of pulp/seed are due to the disruption of integrity and fluidity of the cell wall.

Cytotoxicity of *C. fistula* extracts was evaluated against human intestinal cell line (INT-407) by MTT cell metabolic assays, neutral red (NR), and lactate dehydrogenase (LDH) enzyme leakage assays. LC_{50} of the hexane extract of seed (HES), ethyl acetate fraction of seed (EFS), n-butanol fraction of seed (BFS), hexane extract of pulp (HEP), ethyl acetate fraction of pulp (EFP), and n-butanol fraction of pulp (BFP) treated cells showed 938.60, 759.35, 830.56, 866.89, 714.36, and 826.93µg/ml respectively.

Extracts were further evaluated for free radical scavenging activity to cope with the oxidative challenge induced by hydrogen peroxide (50µM). Treatment of 50µM H_2O_2 on INT-407 cells showed no cytotoxicity, however decreased the activity of Catalase, Superoxide dismutase (SOD), Glutathione peroxidise, and Glutathione reductase by 61.33, 67.99, 62.32 and 15.0% respectively. The treatment of hexane extracts of pulp/seed did not support the free radical scavenging enzyme system to minimize the oxidative challenge, whereas ethyl acetate and n-butanol fraction of seed/pulp showed cell protection by activation of Catalase and Glutathione redox cycle. The activity of Catalase increased in ethyl acetate and n-butanol fraction of pulp/seeds probably due to increase in SOD activity that formed the substrate for the enzyme. Such increase of Catalase activity in ethyl acetate and n-butanol fraction of pulp/seeds prevent the oxidative damages of the cells.

Antiproliferative activity of *C. fistula* extracts were evaluated against breast cancer (MCF-7) and cervical cancer (HeLa, and SiHa) cell lines. All the extracts decreased proliferation of cells along with concentration. LC_{50} of the HES, EFS, BFS, HEP, EFP, and BFP treated HeLa cells showed 755.5, 464.5, 585.6, 740.5, 438.2, and 570.7µg/ml; SiHa cells showed 755.4, 435.7, 580.2, 740.5, 415.5 and 535.3µg/ml and MCF-7 cells showed 792.8, 451.4, 575.6, 735.3, 422.2, and 564.5µg/ml respectively. Among the tested extracts, ethyl acetate fraction of seed/pulp showed lowest LC_{50} when compared with the other extracts. LC_{50} of each extracts against HeLa, SiHa, and MCF-7 cell lines showed similar trend in LDH and NR cytotoxicity assays. LDH cytotoxicity assay significantly correlated with MTT and NR cytotoxicity assays ($p \leq 0.04$). The significant correlation coefficient (r) between the trends of each extracts in NR and MTT assays were more than 0.91. Long term cytotoxicity effects of ethyl acetate fraction of pulp/seed and n-butanol fraction of pulp/seed further confirmed by the clonogenic assays. Ethyl acetate fraction of seed/pulp treated cell lines showed drastic changes in cellular morphology, disassembly of fibers and focal contacts, or inhibited cell spreading as compared to the other tested extracts.

Apoptotic mechanism of cell death further evaluated by means of p53, Bcl2 gene expression; *caspase* enzymes activation and DNA fragmentation. Ethyl acetate and n-butanol fraction of pulp/seed were associated with up-regulation of *p53* and down regulation of *bcl-2* expression and also activates *caspase*s enzymes. Expression level of *caspase-9* and *capase-3, 7 & 10* enzymes were significantly correlated with student's *t* test ($p \leq 0.001$). Apoptotic cell death was further confirmed by the genomic DNA fragmentation of cell lines treated with ethyl acetate and n-butanol fraction of pulp/seed.

GC-MS analysis revealed the presence antimicrobial compound like *β-stigmasterol, β-sitosterol, campesterol, fucosterol,* and *lathosterol* in hexane extract of seed/pulp. Ethyl acetate fraction of seed/pulp also contain some previously reported antiproliferative compounds like *1,8-dihydroxy-3-anthraquinone carboxylic acid,* and *thymol* whereas n-butanol fraction of pulp/seed contain previously reported cytotoxic compounds like *inositol* and *scopolin.*

Present study we concluded that methanolic extract of *C. fistula* pulp/seed has antioxidant as well as free radical scavenging potential that enhanced defensive enzyme system and protect the cells from the harmful effect of environmental toxicants. However, methanolic/hexane extracts might be helpful in protection against fungal infection (candiasis) which is predominant among the immunocompromised patients. Ethyl acetate and n-Butanole fraction of pulp/seed induced apoptosis in cancerous cells might be due to presence of active antiproliferative compounds in these extracts.
