ABSTRACT

Standardization and Biochemical Evaluation Studies on Jatropha tanjorensis – A Traditional Anti-Cancer Drug Source

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Cancer refers to one of the large number of diseases characterized by the development of abnormal cells that divide uncontrollably and have the ability to infiltrate and destroy normal body tissue. It is one of the major causes of mortality in developed and developing countries, with a total of more than 8 million new cases diagnosed every year. Chemotherapy can be quite effective in treating these deadly cancers, but these agents reach all parts of the body, not just the cancer cells. Because of this, there are many side effects reported during treatment such as organ damage and onset of secondary cancers. Hence there is a need of alternate therapy to combat this disease.

Cancer patients often resort to Herbal medicine, one of the most commonly preferred complementary and alternative therapies (CAM). Besides, reducing tumor cell proliferation, herbal medicine also quenches the onset of secondary cancer and other inflammations through immunomodulation by improving antioxidant status, body fluids and physical fitness. Plant derived agents like taxol, vinblastine, vincristine, camptothecin derivatives topotecan and irinotecan, and etoposide are in clinical use all over the world.

Plant families with wide distribution are of major importance and selected for plant-
derived pharmaceutical researches, one such family is Euphorbiaceae which is selected for the present work. Euphorbiaceae is also considered to be one of the medicinally important plant families and have been recently shown that these members possess anticancer, antioxidant, antimicrobial and anti-inflammatory effects. Hence in the present work attempts were made to develop an anticancer herbal drug from a common Euphorbiaceae member available in and around Thanjavur, Tamil Nadu, INDIA. *Jatropha tanjorensis* Ellis & Saroja, is selected and evaluated for its anti cancer potential by subjecting to standardization and validation studies.

Through standardization studies botanical and chemical standards along with HPTLC profiles were determined for the selected *J. tanjorensis* which will be useful in deciding the quality of the herbal drug and to obtain uniform and reproducible results in the preclinical and clinical studies.

Attempts were also made to understand the mechanism of anti-cancer action of the bioactive extract of the selected plant by performing docking studies using the molecules identified in the extract which is subjected to reversed-phase Liquid chromatography with C18 bonded silica gel coupled to ultraviolet (UV) detection and Mass spectrometer encompass of quadrupole mass analyzer with time-of-flight mass detection (micrOTOF-QII). Identified compounds were assigned their structure by comparing their mass signals with compounds already reported and MS/MS spectral libraries. With this approach several compounds not previously reported from *J. tanjorensis* were identified. The methanolic extract from leaves of *J. tanjorensis* (MEJT) contained a complex mixture of flavonoids predominantly as glycosides, with few aglycones such as Vitexin, naringin, isorhamnetin-3-Glucoside-4'-Glucoside, delphinidin-3-O-2''-O-β-xylopyranosyl-beta-glucopyranoside, 6-c-hexosyl-8-c-pentosyl apigenin, cyanidin-3,5-di-
O-glucoside, 3',7-Dimethoxy-3-hydroxyflavone and 2'',3'',4',5,6'',7-Hexa-O-methylisovitexin. The core aglycones of these flavonoids were identified as apigenin, luteolin, isorhamnetin, quercetin and kaempferol. Few other bioactive molecules like rhein, benzamidine and derivative of ellagic acid were also identified. Apart from phenolic compounds few medicinally potent alkaloids like norharman, harmane, salsolinol and anabasine were also identified. To depict the biological activity against cancer cells, inhibition of proliferation assay (MTT) was performed on EAC cells (IC\textsubscript{50} of 14.57 µg/mL), Caco-2 cells (IC\textsubscript{50} of 21.0 µg/mL) and Human skin carcinoma cells (A431) with an IC\textsubscript{50} of 58.53 µg/mL.

MEJT when subjected to microbiological screening showed potent antimicrobial activity against various strains of bacteria with an MIC of 7.8 µg/mL. MEJT was also evaluated for its anti-inflammatory activity through proteinase inhibition activity (IC\textsubscript{50} 166.2 µg/mL), inhibition of protein denaturation (IC\textsubscript{50} 165.4 µg/mL) and membrane stabilization (IC\textsubscript{50} 160.4 µg/mL). Antioxidant property was evaluated using reducing hydroxyl ion assay (with an IC\textsubscript{50} of 46.43 µg/mL) DPPH free-radical scavenging activity (IC\textsubscript{50} of 49.7 µg/mL) and lipid peroxidation inhibition activity (IC\textsubscript{50} of 189.6 µg/mL).

Results acquired proved the potent efficiency of selected plant against various disease conditions progressing to cancer. Present study also provides necessary information on the chemical composition of \textit{J. tanjorensis} leaves, thus suggesting yet another “authentication” through chemical constituents in deciding the genuineness and quality of the selected plant. Present study also revealed that MEJT showed significant antitumor, antioxidant and immunomodulatory activities in EAC bearing mice. The dose dependent reduction in body weight, tumor volume, packed cell volume, tumor cell counts and increase in mean survival time (MST) and percentage increase in life span (upto 300%) in
MEJT treated animals were observed. There was a significant increase in RBC count; Hb content in MEJT treated animals along with a reduction in total WBC count was observed. A significant increase in total protein and albumin content, but a decrease in AST, ALT and ALP contents were also noticed in MEJT treated animals. A significant decrease in liver MDA levels and increase in Catalase and reduced glutathione levels were observed in MEJT treated animals. The results were more significant in mice treated with 400 mg/kg body weight. Furthermore, IL-1 beta, IL-6, TGF-beta levels decreased significantly and a significant increase in TNF-alpha amounts were observed in MEJT treated animals. Results concluded that the MEJT was effective in inhibiting the tumor growth in ascitic tumor models. The biochemical and antioxidants studies also supported its antitumor properties. The data of the results suggested that MEJT induced apoptosis through Bcl-2/Bax pathways, where MEJT has increased the expression of Bax and decreased the levels of Bcl-2. A decrease in VEGF level also suggests its antiangiogenic activity. Present study thus depicted that J. tanjorensis has a potential to be exploited further for the search of a human friendly anticancer/antitumor drug with high nutraceutical potentials.