Research Article
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In-vitro cytotoxicity studies on methanolic leaf extract of *embelia ribes* burm f - an important traditional medicinal plant of Kerala

Abstract

Medicinal plants play a key role to cure many diseases from time immemorial. The usage of medicinal plants in traditional medicinal system is the vital process of India. Cancer is one of the killing diseases and causes severe defects on human being. There are many types of cancer diseases in human beings affects the different organs. There is no proper medicine to cure such kind of cancer diseases. In the present study the in-vitro cytotoxicity potential of methanolic leaf extract of Embelia ribes Burm F. was carried out against three cell lines U87, HepG2 and MCF7. The results revealed that the cytotoxicity potential of the leaf was increased when the concentration of leaf extract increases. Among the three cell lines the highest percentage of growth inhibition was observed against U87 cell lines. Based on the results, the leaf of the plant can be used to prepare anticancer drug with proper standardization methods.

Keywords: embelia ribes, cytotoxicity, cancer cell lines, medicinal plant

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Abbreviations: U87, uppsala 87 (brain cancer cell line); Hep G2, liver hepatocellular carcinoma (human liver cancer cell line); MCF 7, michigan cancer foundation-7 (breast cancer cell line); *E. ribes, embelia ribes*; Gm, gram; Ml, milliliter; µg, micro gram; µl, microlitre ; °C, degree celsius; Rpm, revolutions per minute; DMSO, dimethyl sulfoxide; No, number; %, percent; U/ml, unit per milliliter; FBS, fetal bovine serum; pH, potential of hydrogen; MTT, 3-(4,5-di-methylthiazol-2-yl): 2,5-diphenyl tetrazolium bromide; A570, absorbance at 570nm; M, molar concentration; HCl, hydrochloric acid; CO_2, carbondioxide; COLO-205, colon cancer cell lines; K562, human leukaemic cells; DLA, dalton's lymphoma ascites cells; BIR, baculovirus iap repeat; XIAP, x-linked inhibitor of apoptosis protein; COLO, 205 - colon cancer cell lines; LC, lethal concentration

Introduction

Cancer is the leading killing disease after the cardiovascular disorders. There is no proper medicine for controlling the growth of the cancer cells. There has long been standing interest in the identification of natural products for the treatment of various diseases for thousands of years. Natural products possess immense pharmacological significance in the development of drugs including cancer.[1–3] The majority plant derived phytoconstituents, such as paclitaxel, etoposide, camptothecin, vinca alkaloids, indole alkaloids, podophyllotoxin derivatives, etoposide and teniposide, currently used in clinical cancer chemotherapy. The efficacy of chemotherapy, radiotherapy, hormonal therapy, or surgery, which are mainly used for the treatment of cancer, are well-known for side effects;[4] hence, the identification of novel natural products that possess better effectiveness against cancer, but less harmful effects have become desirable,[5] and therefore, natural products are continuously being explored worldwide.

E. ribes is widely used as traditional herbal medicine in India. The plant is a climber with slender branches and long internodes. The leaves are elliptic, broad and covered with minute glands. The flowers are small, white racemes arranged in panicle inflorescence at the end of the branches. The fruits are berries, round, red to black color and tipped with style.[6] In Indian system of medicine 'Ayurveda', the plant is popularly known as Vidanga or Bashmak or Krimigna (Sanskrit); Baberangor Wawrung (Hindi); Vayuvilanga (Kannada) and it is used as one of the adjuvant in most of the drug preparations. The whole plant is used in the treatment of anti-inflammatory to relive rheumatism and fever.[7] The fruit is bitter in taste, good appetizer, cures tumors, ascites, bronchitis, jaundice and mental disorders.[8] Seeds are used as antibiotic, anthelmintic, anti-tuberculosis, alterative and stimulative.[9] Leaves are astringent, demulcent, depurative and useful in pruritus, sore throat, and ulcers of mouth, indolecent, skin diseases and leprosy.[10] All the parts of this plant have the enormous medicinal properties. So the present study framed to investigate the *in vitro* cytotoxicity potential of leaf extracts of *E. ribes*.

Materials and methods

The leaves of the *Embelia ribes* Burm. F. was collected from Kakkayam, Kozhikode, Kerala and the plant material was identified by the experts at M. S. Swaminathan Research Foundation and also by literature survey.

Preparation of plant extract

The collected plant leaves were cleaned and shade dried for a week. 10gm of pulverized leaf material was mixed with 100ml of methanol and kept in a rotary shaker at 100rpm overnight and filtered with Whatman no. 1paper and concentrated to dryness at 40°C, lyophilized and stored at 4°C until further use. Different concentrations of the methanolic extracts (0.4, 2, 10, 50 and 250µg/ml) were prepared in 0.5% DMSO for determining cytotoxicity.

Cell line and culture condition

U87 (brain cancer cell line), HepG2 (human liver cancer cell line) and MCF7 (breast cancer cell line) were used for the *in-vitro* cytotoxicity studies. The cells were maintained in Minimal Essential Media supplemented with 10% FBS, penicillin (100U/ml), and

streptomycin (100µg/ml) in a humidified atmosphere of 50µg/ml CO_2 at 37 °C.

In vitro assay for cytotoxicity activity (MTT assay)

The Cytotoxicity of samples U87 (brain cancer cell line), HepG2 (human liver cancer cell line) and MCF7 (breast cancer cell line) were determined by the MTT assay.[11–14] Cells (1×10^6/well) were plated in 1ml of medium/well in 24-well plates. After 48hours incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations (0.4µg/ml, 2µg/ml, 10µg/ml, 50µg/ml and 250µg/ml) of the leaf extract of *Embelia ribes* in 0.5% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide cells(MTT) phosphate- buffered saline solution was added. After 4h incubation, 0.04M HCl/isopropanol were added. The absorbance at 570nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. MTT assay is a quantitative colorimetric assay for measuring cellular growth, cell survival and cell proliferation based on the ability of living cells. The assay was carried out using (3-(4,5-dimethyl thiazol-2yl) - 2,5-diphenyl tetrazolium bromide (MTT). MTT is cleaved by mitochondrial enzyme dehydrogenase of viable cells, yielding a measurable purple product formazan. This formazan production is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity.[15–18] Triplicate analysis of *in-vitro* cytotoxicity of leaf extracts of *Embelia ribes* was carried out with various concentrations.

The effect of the samples on the proliferation of cell lines were expressed as the % cell viability, using the following formula:

$$\%\ cell\ viability\ = \frac{A570\ of\ treated\ cells}{A570\ of\ control\ cells} x100$$

Statistical analysis

The data are expressed as mean±standard deviation (SD) for at least three independent determinations in triplicate for each experimental point. The percentages of cell growth were used to obtain the full dose response curves and to determine the LC50 values (Lethal concentration inhibiting 50% of the cell growth compared with control). The data was analysed using Probit Analysis of SPSS package.

Results and discussion

In the present study, *in-vitro* cytotoxicity effects of leaf extracts of *Embelia ribes* was carried out with various concentrations for the following cancer cell lines U87 (brain cancer cell line), HepG2 (human liver cancer cell line) and MCF7 (breast cancer cell line). The leaf of the plant was collected from Kerala and shade dried powdered and extracted with methanol solvent. Five different concentrations (0.4µg/ml, 2µg/ml, 10µg/ml, 50µg/ml and 250µg/ml) of leaf extracts were used to study the cytotoxicity potential of the plant. The ctytotoxicity potential of various concentrations of methanolic extracts with LC 50 and LC 90 values of *Embelia ribes* was displayed in Table 1 & Figure 1. The results revealed that the cytotoxicity rate was increased when the concentrations of leaf extract increases. MTT assay measured the cell viability based on the reduction of yellow tetrazolium MTT to a purple formazan dye mitochondrial dehydrogenase enzyme. So, the amount of formazan produced reflected the number of metabolically active viable cells. Among the three cell lines U87 have the highly potent activity followed by MCF7 and HepG2. But in HepG2 cell line, the concentration of 250µg/ml revealed more activity than MCF7 cell line. The LC50 and LC 90 values observed for Cell line U87 against methanolic extract of *Embelia ribes* was 13.36µg/ml and 127.98µg/ml. The values observed in cell lines HepG2 and MCF7 were 85.58µg/ml, 49.98µg/ml and 222.79µg/ml, 235.79µg/ml respectively for LC50 and LC 90 concentrations.

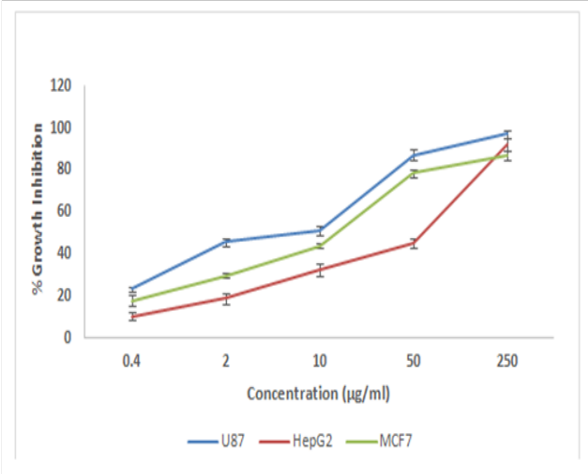

Figure 1 Percentage of cell growth inhibition of various concentrations of *Embelia ribes* against three cell lines.

The plant has the enormous medicinal properties and its various parts used to cure many diseases. Embelin, a naturally occurring quinonoid compound, is found to be the major constituent of *Embelia ribes*.[19] Embelin, is the anti-cancer compound, so the plant exhibited the potent cytotoxicity against cancer cell lines. Embelin is reported to decrease tumor size and inhibit the increase in activity of serum enzymes, viz. acid phosphatase, τ-glutamyl transferase, lactate dehydrogenase, aldose, etc in rats with experimental fibrosarcoma. Embelin interferes with carbohydrate and amino acid metabolism in tumor bearing animals.[20,21] Reported that the crude hexane extract of the fruits of *Embelia ribes* exhibited cytotoxicity against Human leukaemic cells (K562) and Dalton's Lymphoma ascites cells (DLA). *In-vitro* studies on Embelin suggest the potential of the compound on those two cell lines. However the compound did not exhibit toxicity on normal lymphocytes isolated from human blood preferentially attacking the tumour cells. Many reports revealed the anticancer potential of *Embelia* species, which may be due to the presence of phytoconstituent Embelin in this plant. Chitra et al.[22] reported antitumor activity of embelin in methylcholanthrene induced fibrosarcoma in albino rats and in addition enhancing their survival time. Nikolovska-Coleska et al.[23] reported embelin as a fairly potent, nonpeptidic, cell-permeable, small-molecule inhibitor of XIAP and represents a promising lead compound for entirely new class of anticancer agents that target the BIR3 domain of XIAP. Dai et al.[24] reported embelin inhibits chemical carcinogen-induced colon carcinogenesis.[25] studied the *in-vitro* cytotoxic activity of methanolic extract of *Embelia tsjeriam* against human breast (MCF7) and colon cancer cell lines (COLO-205) using the Sulfarhodamine B assay. The methanolic extracts inhibiting at least 50% of tumor cell proliferation at dose of 6.25- 400µg/ml. MCF7 cell lines also inhibited (15- 250µg/ml)by methanolic extract of *E. ribes* in the present study.

Table 1 Cytotoxicity effect of various concentrations of Embelia ribes against three cell lines

Cell lines	0.4µg/Ml	2µg/Ml	10µg/Ml	50µg/Ml	250µg/Ml	Lc50 µg/Ml	Lc90 µg/Ml
U87	23.3±1.27	45.6±1.91	51.5±2.23	87.2±2.56	97.2±1.89	13.36	127.98
HepG2	10.5±1.97	18.8±2.43	32.3±3.01	45.0±2.2	92.3±2.96	85.58	222.79
MCF7	17.9±2.67	29.6±1.1	43.87±1.03	78.5±2.03	87.0±1.98	49.98	235.79

Conclusion

Embelia ribes is one of the potent medicinal plants used in Traditional medicinal systems of India. All the parts of the plants have the potent medicinal property and used to cure many diseases. The current research also added one more potent activity of the leaf of the plant. The further research is necessary to design the drugs for cancer diseases in pharmaceutical industries.

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Conflict of interest

The author declares no conflict of interest.

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