Original Article

Extracts of Jamun seeds inhibited the growth of human (Hep-2) cancer cells

ABSTRACT

Introduction: In the last century, the human laryngeal epithelioma has become a life-threatening disease leading to a high rate of mortality worldwide. The current investigation is focusing on the antiproliferative effect of *Eugenia jambolana* seed extracts against Hep-2 cancer cells.

Methods: The active compounds from the seeds of *E. jambolana* were extracted by the decoction extraction method using acetone, ethanol, and methanol. The filtrates from the different solvents were subjected to liquid-liquid separation before drying by a rotary evaporator. In various doses, the crude extracts and carcinoma were subjected to a methylthiazolyl diphenyl tetrazolium bromide assay. Cell viability was determined under ultraviolet visualization at an absorbance of 540 nm. The data of the viable cells were subjected to analysis of variance at $P \leq .01$.

Results: Crude compounds of *E. jambolana* seeds extracted by acetone, methanol, and methanol extract had an anticarcinoma effect. Among the extracts, methanol extract possessed a recommendable anti-carcinoma effect compared to acetone and ethanol crude extracts. At a concentration of 125 μg/mL, the crude extracts of methanol, acetone, and ethanol destroyed 49.57, 35.01, and 27.67 carcinomas, respectively. The concentration of 31.25 μg/mL of acetone extract and 125 μg/mL of ethanolic extract affected 28.11 and 27.67 carcinomas, respectively.

Conclusions: *E. jambolana* seeds possess anticarcinoma potency and thus can be administered in the reduction of proliferative carcinoma. The study recommended further studies which will involve the elution of pure compounds from the methanol extract of *E. jambolana* that possess antitumour and antiproliferative activity against Hep-2 cell lines.

KEY WORDS: Anti-carcinoma; *Eugenia jambolana*; Human laryngeal epithelioma type 2 (Hep-2) cells

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INTRODUCTION

Herbal medicine and the healing potential of plants have been known and used since 3000 BC.[1] The medicinal use of plants and their products has been passed down in several generations of history that have significantly led to the development of modern medicine systems.[2,3] As per reports by the World Health Organization, it is estimated that still 82% of the population of developing countries still depend on traditional medicinal plants as a source of the drug.[4] Plants produce a whole series of different compounds in the form of secondary metabolites which are not of particular significance for primary metabolism but are biochemically beneficial to various target sites of the mammalian body.[5] Consumption of plant parts by herbivores and human delivers secondary metabolites into the circulatory system; these compounds turn to be biologically active substances and have remarkable health and pharmacological effects in the body in a direct or indirect mechanism.[6,7] Compounds of polar and bipolar nature that are found in plant tissues are easily extracted in the laboratory by soaking in chemical solvents like propane, acetone, and methanol; these compounds contain active portions that have a specific target and function in human health.[8]

Eugenia jambolana Lam. commonly known as miracle fruit, jambul, and black plum belongs to the member of the family Myrtaceae and is highly dominant in India, South-East Asia, and Eastern Africa.[9] *E. jambolana* has been reported to contain chemical compounds like coumarins, flavonoids, glycosides, phenols, tannins, and steroids which are used in various therapeutic applications like

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the treatment of diabetes and skin ulcers.[10,11] Scientifically, the noncommunicable disease, cancer, specifically carcinoma has been announced as the second leading death cause after cardiovascular disease.[12] More than 22 million people are diagnosed with cancer worldwide yearly; among them about 10 million people are carcinomic and more than 6 million die of carcinoma in a year.[13]

Carcinoma is categorized as a complex disease due to its association with a wide range of effects at both molecular and cellular levels.[14] Carcinomas attack the Human Laryngeal Epithelioma Cells (Hep-2 cells) located in the skin, lungs, and breast, where it proliferates, and if not treated, the cells undergo metastasis, thus leading to the death of a person.[13] When a patient is diagnosed with carcinoma, he is subjected to surgery, therapy by radiation, and chemotherapy to kill or remove the cancer cells; this kind of treatment is costly especially for low-income earners.[15] Scientists are intensively investigating various methods of mitigating and treating carcinoma. Recently, there has been an increase in studies exploring extracts from natural products, especially of plant sources, for their anticancer activity on cell lines.[2,5] This research was focused on determining the viability of *E. jambolana* extracts on the carcinoma of Hep-2 cells.

MATERIALS AND METHODS

Sampling and identification

Fresh fruits of *E. jambolana* were identified and harvested from Pallipalayam in Namakkal district (11° 21' 52" 62°N, 77° 44' 52" 52°E altitude of 165 m above sea level). Morphological identification was done at the herbarium of the Botany Department at Periyar University. The fruits were properly washed with tap water before excavating the seeds from the flesh. The seeds were left to dry for one month under shade in a well-ventilated area in the microbiology laboratory of the Department of Microbiology at Periyar University. The dry *E. jambolana* seeds were macerated using a mechanical grinder and passed through a 40-μm mesh sieve to collect the fine powder. The *E. jambolana* seed powder was stored in airtight containers at room temperature with 10 ± 2% moisture content.

Ethical clearance

This study was approved by the Department of Physical and Biological Sciences of Bomet University College and the Health Ethical Committee of Kings Institute of Preventive Medicine, no.131/KIPM/PBS/2021, on January 10th, 2020.

Cell culture

The carcinomas and normal human laryngeal epithelioma cells (Hep-2 cells) were obtained from the King Institute of Preventive Medicine, Chennai, India. The cells were maintained in minimal essential media (MEM) supplemented with 10% fetal bovine serum, penicillin (100 μg/mL), and streptomycin (100 μg/mL) and placed in a humidified atmosphere of 50 μg/mL CO_2 at 37°C in the microbiology laboratory at Periyar University for experimentation. The analytical grade solvents; acetone, ethanol, methanol, the MEM, fetal bovine serum, trypsin, 3-(4, 5-dimethyl-2thiazolyl)-2, 5-diphenyl--tetrazolium bromide, and dimethyl sulfoxide (DMSO) were purchased from Salem Scientific Suppliers at Salem district, India.

Preparation of E. jambolana crude extract

Acetone, methanol, and ethanol were used to extract the crude active compounds from the *E. jambolana* seed powder by the decoction extraction method as described by Tandon and Rane.[16] This involved subjection of 5 g powder in 100 mL of the solvent in a sterile conical flask and allowed to heat at 40°C in a water bath for 48 hours. The mixture was passed through a Whatman No. 1 filter paper to obtain the filtrate. The filtrate was subjected to liquid-liquid separation in a separating funnel, and this involved the filtrate with an equal amount of distilled water. The obtained mixture in the supernatant was subjected to dryness by the rotary evaporator at 40°C and stored in the refrigerator at 4°C as active powder in sterile, capped, and well-labeled bottles. The dry active crude compounds were reconstituted by redissolving in 0.1% DMSO in different concentrations of 8, 15.6, 31.25, 62.5, 125, 250, 500, and 1,000 μg/mL for experimentation of the anticancer activity.

Methyl-thiazolyl diphenyl-tetrazolium bromide assay

The carcinomas (1×10^5 cells/well) were plated in 0.2 mL of MEM medium/well in a 96-well plate using a micropipette; 0.2 mL of 8, 15.6, 31.25, 62.5, 125, 250, 500, and 1,000 μg/mL concentrations of the acetone extract were added in the wells. Each concentration was placed on 12 wells. The cells were then incubated in 5% CO_2 for 24 hours. After incubation, the cells were washed with 10 μL/well of phosphate-buffered saline (pH 7.4), and then the cells were subjected to 20 μL/well of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5 diphenyl-tetrazolium bromide (MTT) in phosphate-buffered saline solution (5 mg of MTT/mL of buffer saline). The washed cells were again subjected to incubation for 4 hours before the addition of 1 mL of 0.1% DMSO. This procedure was used for both ethanol and methanol extracts. On a different plate, normal Hep-2 cells (1×10^5 cells/well) were plated in 36 wells with 0.2 mL of MEM medium/well. Using the above procedure, 0.2 mL of acetone extract (125 μg/mL of 0.1% DMSO) were tested as positive controls on 12 wells, as the remaining 24 wells were used by ethanol and methanol extracts as their positive control. On the same plate, 12 wells were plated with carcinomas (1×10^5 cells/well) and left untreated with neither acetone, ethanol, nor methanol extract, thus acting as a negative control. The cells were subjected to ultraviolet visualization at an absorbance of 540 nm to determine their viability.

Statistical analysis

All data were recorded, converted to percentages, and their means were compared by Duncan's New Multiple Range Test before subjection to one-way nonparametric

analysis of variance (Kruskal-Wallis H test) to determine the significant differences between the viability of cells in the treatment by different concentrations of *E. jambolana* seed crude extracts using SPSS software, version 20. The analysis included comparing the means of the viable cells at a 99% level of confidence using the Tukey HSD test at $P \leq .01$. The independent variables in this model were the dose of the extracts applied and the duration of exposure of the cells to the doses and the percentage of viable cells was the dependent variable.

RESULTS

The results obtained in this study showed that the compounds found in *E. jambolana* seeds possess anticancer activity; this was observed under ultraviolet light on the cells treated with acetone, ethanol, and methanol crude extracts. Apoptosis of the carcinomas increased with the increase in the dosage of the crude extracts as observed in Figure 1.

Tabulated results showed that *E. jambolana* crude extract by methanol possessed a recommendable anticarcinoma effect compared to acetone and ethanol crude extracts. At a concentration of 125 µg/mL, the crude extracts of methanol, acetone, and ethanol destroyed 49.57, 35.01, and 27.67 carcinomas, respectively [Table 1]. Statistical results showed that 8 µg/mL of methanol, 15.6 µg/mL of acetone, and 31.25 µg/mL of ethanol crude extracts affected 16.31, 16.14, and 15.04 carcinomas, respectively, at $P = .0052$. Results showed that *E. jambolana* crude compounds extracted by acetone had a more reliable performance than the ethanol extracts. The concentration of 31.25 µg/mL of acetone extract and 125 µg/mL of ethanolic extract affected 28.11 and 27.67 carcinomas, respectively, at $P = 0.007$.

Table 1: Deceased cells (%Mean±S.E.) after treatment with acetone, ethanol, and methanol extracts of *E. jambolana* seed

Conc. (µg/mL)	Crude compound extract		
	Acetone	Ethanol	Methanol
8	10.09±41.10^{b}	6.22±15.50^{a}	16.31±12.72^{c}
15.6	16.14±8.42^{c}	13.39±9.72^{c}	24.29±9.63^{e}
31.25	28.11±62.0^{f}	15.04±21.3^{c}	36.56±14.01^{h}
62.5	32.46±14.39^{g}	20.0±36.90^{d}	40.88±3.34^{i}
125	35.01±33.83^{g}	27.67±4.61^{f}	49.97±10.09^{k}
250	49.08±6.67^{k}	38.16±99.10^{h}	62.61±8.21^{n}
500	51.24±44.22^{l}	44.87±77.83^{j}	75.13±11.80^{o}
1,000	64.8±3.75^{n}	57.44±63.32^{m}	83.61±64.22^{p}

Means in the table that do not share a letter are statistically different from each other (n=288), ($P \leq 0.01$) using Tukey's HSD test.

DISCUSSION

Despite a good understanding of the molecular basis of carcinoma and advances in treatment by chemotherapy and radiotherapy, it remains a global threat and a major cause of death.[12] MTT assay was used in this study; various research studies have considered MTT assay as a reliable assay to determine the extent of carcinoma viability.[17] In this study, anticancer activity in the form of dose-dependent was observed in the *E. jambolana* ethanolic extract; this was also reported by Ebrahimi *et al.*[7] on the ethanolic extract of *Pleurotus ostreatus* which repressed the proliferation of leukemia cells (HL-60 cell line). This study recorded the destruction of 6.22 carcinomas by 8 µg/mL of ethanolic extract; similar studies by Rady and Bashar showed anticarcinogenic activity by the ethanolic extract of *Negombata magnifica* by MTT assay where 12.5 µg/mL suppressed 4.68 of the liver carcinoma (HepG-2 cell line).[18]

This research recorded that the methanolic extract of *E. jambolana* seeds had an anticarcinoma activity. Studies by Siddika *et al.*[19] recorded anticarcinogenic properties of the methanolic extract of *Syzygium cumini* on the subcutaneous Ehrlich ascites carcinoma. A death of 49.97 carcinomas was caused by 125 µg/mL of the methanol extract of *E. jambolana*; similar studies by Gong *et al.*[20] recorded a significant cytotoxic effect of 24.28 human leukemic cell lines (HL-60) caused by 125 µg/mL of the methanol extract of *Bichofia javanica* leaves. Despite this research showing the anticarcinoma activity of the methanolic extract of *E. jambolana* seeds in the Hep-2 cells, El-Shenawy, Kujawska, and Jodynis also reported that the administration of the *E. jambolana*–grounded seed powder at 25 mg/kg body weight/day was effective in the prevention of colon carcinogenesis.[10,21]

CONCLUSION AND RECOMMENDATIONS

This study has shown that *E. jambolana* seeds possess anticarcinoma potency and thus can be administered in the

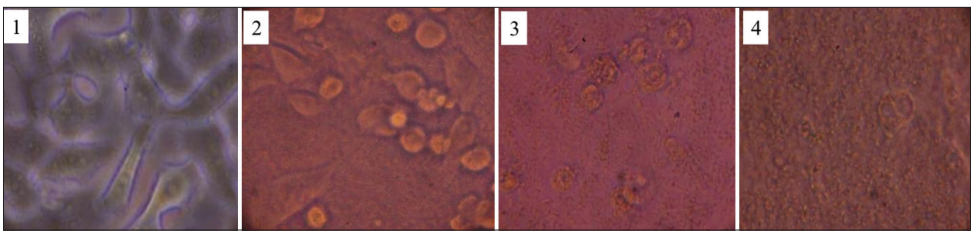

Figure 1: Viable and dead Hep-2 cells after MTT assay with *E. jambolana* seed extract. (1) Positive control (Normal Hep-2 cells) treated with 125 µg/mL of methanol extract; (2) Carcinomas treated with 8 µg/mL of methanol extract; (3) Carcinomas treated with 125 µg/mL of methanol extract; and (4) Carcinomas treated 1,000 µg/mL of methanol extract

reduction of proliferative carcinoma on Hep-2 cell line. The study recommended further studies that involve the elution of pure compounds from methanol extract of *E. jambolana* that possess antitumor and antiproliferative activity against cancer cells. The determination of the LD and LC_{50} of the active compound on carcinoma and a further investigation on the mechanism of action of the pure bioactive compounds on carcinomas.

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Conflicts of interest

There are no conflicts of interest.

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