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Antitumor Activities of KALI Banana Fruit in MG-63 Cell Line

Research Article

Anu A and Geethalashmi S*

Department of Biotechnology, Sree Narayana Guru College, Coimbatore, India

***Corresponding author:** Geethalasmi S, Associate professor, Department of Biotechnology, Sree Narayana Guru College, Coimbatore, India; E-mail: s.geethalakshmi@gmail.com

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Abstract

Banana natural products are broadly utilized for human consumption. It is the second real nourishment crop in India. There are around 365 varieties of bananas available throughout the world. It is utilized as conventional medication for bowel disorders. This study aimed at examining the anticancer activity of Kali variety of banana which is commonly consumed in southern part of India. Anticancer examination of the sample was performed for the ethanolic extract using MG-63 cell lines which showed a high degree of anticancer activity which was proved by the cytotoxic effect on MG-63 tumor cell lines. Phytochemicals present in the plant concentrate provoked cell apoptosis and smoother cell expansion to quickly partitioning malignancy cell lines. The kali variety shows increased level of anti cancer activity in both cell lines. So we can use this variety in oral medicine for cancer treatment.

Keywords: MG-63; Kali banana fruit; Bioactive compounds; Apoptotic index; Phenols; Flavanoids

Introduction

Malignancy is a strange cell development with the potential to spread to other parts of the body (WHO, 2018). According to ASCO information, men have a high occurrence rate of bone marrow cancer than women. Genetic disorders, hereditary, exposure to toxic chemicals like solvents, fuels, certain cleaning products, radiation cause bone marrow cancer in human. There are 1660 death (960 men and 700 women) from this disease in 2018. A huge number of patients show resistance to tamoxifen, the currently available high potent drug for treating bone marrow cancer and experience extreme reactions. Other treatment methods like chemotherapy, radiation and bone marrow transplantation result in serious side effects which complicate the current situation. So, medical research focuses on natural methods to cure cancer without additional side effects. Numerous fruits like guava, banana [1], papaya, apple, watermelon, litchi have been accounted to have demonstrated therapeutic properties in several studies [1-6].

Kali is a local name of kali vazha which is a traditional variety of banana in South India. It is a wild type of banana and it cultivated in hill area in Kerala. This variety is used as traditional medicine in Kerala. The fruit occur as a bunch weighing 6-8 kg. Presently, this variety is endangered because of climatic change and modernization of agriculture techniques. The kali fruit is a rich source of crude protein (6-7%), crude fat (9-12%) and total ash (7.6%). Natural bioactive compounds such as steroids, flavonoids, tannins, phenol, Saponins, alkaloids, glycosides are found in the mature fruit. Pieces of banana have for some time been utilized in customary medication in Asia and Africa for treating wounds, decrease pain, for cell rejuvenation, antimicrobial agent and many more [4,6-9].

This study has been aimed at analyzing the anticancer activity of Kali fruit with reduced risk [5,8-12]. The present investigation was directed to profile the bioactive compounds in kali pulp and to evaluate its ability of cancer prevention and analyze its capacity to hinder the expansion of human bone osteosarcoma cells using MG-63 cell line.

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Materials and Methods

Kali fruit was collected from Attapady, Palakkad district, Kerala, India. The peel was removed from fruit, the pulp was collected, shade dried at room temperature for 40 days, powdered and stored at 4 °C until further use.

Preparation of plant extract

Ten gram of dry fruit powder was dissolved in 100 ml of hexane in a conical flask and kept for 24 hours in a shaker. The mixture was then filtered twice using Whatman No. 1 filter paper and stored at 4°C.

Qualitative analysis of secondary metabolites

GC-MS investigation of plant concentrates was performed using Shimadzu GC-MS hardware of model QP 2010S containing Rxi-5 ms combined silica narrow section of 30 m length, 0.25 mm distance across and film thickness of 0.25 µm. This analysis was done to find the metabolites present in the extract.

Cell line maintenance

Human MG63 cell lines were obtained from National Center for Cell Sciences (NCCS), Pune, India. Dulbecco's Modified Eagle Media (DMEM) was utilized for maintaining the cell line, which was enhanced with 10% Fetal Bovine Serum (FBS). Penicillin (100 U/ml) and streptomycin (100 µg/ml) were added to the medium to prevent bacterial contamination. The medium with cell lines was kept in a humidified environment with 5% CO_2 at 37 °C.

Cytotoxicity assay

The MG-63 cells were placed in 24 well plates (1 X 105 cells per well) and incubated in 5% CO_2 environment at 37 °C. Cells (1X105/well) were placed in 24-well plate and incubated in 37 °C with 5% CO_2 condition. Once the cells reached confluence, the prepared concentrations of extract (25 - 300 µg/ml) were added and kept in incubator for 24 hours. Then the samples were removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 0.5% of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-Tetrazolium Bromide (MTT) was added to each well (100 µl/well) and incubated for 4 hours. Then 1 ml of Dimethyl Sulfoxide (DMSO) was added in all the wells to dissolve the formazan crystals. Each sample was placed in the cuvette; using DMSO as the blank the absorbance value at the wavelength of 570 nm was noted using Ultra-Violet (UV) Spectrophotometer in triplicate. The observed values were tabulated and the concentration required for 50% inhibition (IC_{50}) was determined graphically. The percentage cell viability was calculated by determining the ratio between treated MG-63 cells and control multiplied by 100.

Result and Discussion

The hexane extract of the fruit was analyzed for secondary metabolites using GC-MS analysis and the chromatogram and the list of bioactive compounds present in the extract (Figure 1 and Table 1).

The GC-MS chromatogram showed an intense peak at RT 9.376, a second large peak at RT 13.246 and least peak at RT 19.422. The larger peak showed a mass by charge ratio of 155.10 (Figure 1). This m/z ratio does not correlate with any of the prior revealed anticancer bioactive mixes as per the verification in anticancer database and thus has all the earmarks of being a novel one.

The anticancer properties of the pulp with its tumor necrosis factor activity were already reported by many researchers [4,12,13]. MG-63 cell line is a model system for bone cancer studies and apoptosis process in cell. As per literature cited, with a high concentration of bioactive compounds like phenols and flavonoids, high intense anticancer activity can be observed on MG-63 cells [14-18]. Many treatments are used currently in cancer treatment like chemotherapy which, at primary stage, gives good response but it creates resistance in the later stage of cancer. In the present study, different concentration of fruit extract showed various levels of inhibitory action on MG-63 cell lines. Increase in concentration of the extract from 25 µg /ml to 300 µg /ml showed a decrease in cell viability of MG-63 from 90.14 to 26.7 % (Figures 2 and 3). The IC50 value of the fruit extract was found to be 170 µg/ml [19-21].

Conclusion

From the present study, it is clear that the hexane concentrate of kali fruit extracts has a strong cytotoxic activity against MG-63 bone cancer. The fruit have broad spectrum of bioactive compounds which is anticancer active, which is seen from GC-MS chromatogram. Further research is needed to distinguish the bioactive compounds in banana, their refinement and interpretation of their activity to utilize the fruit pulp in pharmaceutical industries.

Table 1: Bioactive Constituents of kali fruit extract.

Peak	Retention time	COMPOUND	M.W. (Daltons)	Area %	Molecular formula
1	7.917	4H-pyran-4-one,2,3-Dihydro-3,5-Dihydroxy-6-Methyl	101.05	11.99	$C_6H_8O_4$
2	8.248	Ethyl caprylate	88.10	1.82	$C_{10}H_{20}O_2$
3	9.252	Coumaran	120.10	1.91	C_8H_8O
4	9.376	5-Hydroxymethylfurfural	97.05	30.09	$C_6H_6O_3$
5	9.708	3-Methoxycatechol	140.05	7.25	$C_7H_8O_3$
6	11.086	Ethyl caprate	88.05	0.72	$C_8H_{16}O_2$
7	11.230	5H-Imidazole-4-Carboxylic acid,5-Amino,Ethyl Ester	155.10	0.98	$C_4H_5N_3O_2$, $C_6H_{13}N_3O_2$
8	13.246	Trimethylolnitromethane	57.00	21.32	$C_4H_7N_4O_{11}$
9	16.048	3-Deoxy-d-mannoic lactone	57.00	20.75	$C_6H_{10}O_5$
10	19.422	Ethyl Palmitate	88.05	0.68	$C_{18}H_{36}O_2$
11	30.272	Glycerol.beta-palmitate	57.05	2.48	$C_3H_8O_3$, $C_{16}H_{32}O_2$

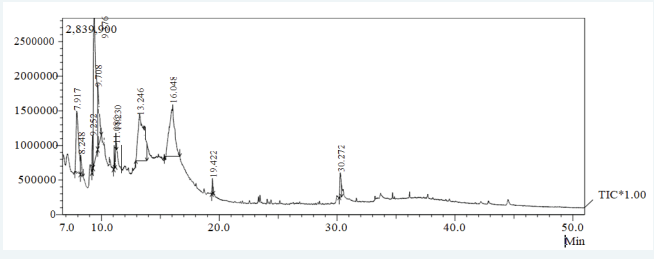

Figure 1: GC- MS Chromatogram of Kali hexane extract.

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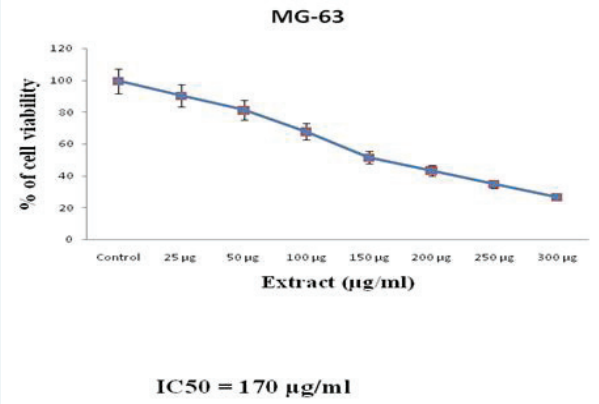

Figure 2: Cytotoxicity assay indicating IC50 value.

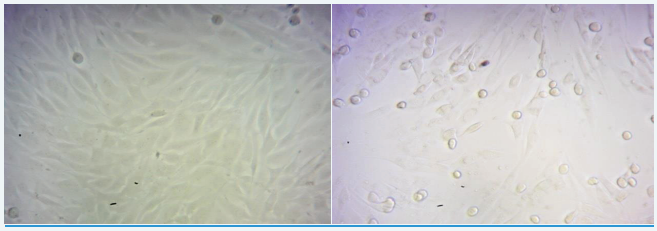

Figure 3: Effect of kali fruit extract after the MTT treatment.

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