Cytotoxic potential of few Indian fruit peels through 3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide assay on HepG2 cells

Munish Garg, Kusum Lata, Saurabh Satija

ABSTRACT

Objective: To investigate in vitro anticancer activity of a few Indian fruit peels through 3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against HepG2 cells.

Materials and Methods: Hydroalcoholic extracts were prepared of five fruit peels, i.e., banana, lemon, guava, orange, and papaya by maceration and thereafter subjected for MTT assay to evaluate anticancer potential on HepG2 cells. Plant extract showed best activity was further fractionated with petroleum ether, chloroform, and ethyl acetate successively and screened again. Phytochemical analysis was then carried out to find out responsible components for the observed activity.

Results: Out of the 40 samples from five fruit peel extracts with rich folklore usage, papaya extract showed maximum activity with least inhibitory concentration \( IC_{50} \) value of 18.5 \( \mu g/ml \). Further analysis after fractionation of the papaya peel extract, aqueous fraction showed the maximum inhibitory activity with least \( IC_{50} \) value of 17.3 \( \mu g/ml \). Phytochemical analysis of the aqueous fraction of papaya peel extract revealed the presence of flavonoids and glycosides. Total flavonoid content found to be 72.25 mg/g.

Conclusion: Papaya fruit extract demonstrated the best activity against MTT assay which may be due to the presence of flavonoids.

KEY WORDS: 3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide assay, Anticancer activity, Carica papaya, fruit peels

Introduction

Cancer is a hyperproliferative disorder which involves transformation, dysregulation of apoptosis, proliferation, invasion, angiogenesis, and metastasis. Malignant cancer is the second leading cause of death worldwide killing about 3.5 million people annually all over the world. Multidisciplinary scientific research attempts are making the best efforts to combat this dreadful disease, but the sure-shot, perfect cure is yet to be brought into medicinal world.\(^1\) There is a continuing need for development of new anticancer drugs through methodical and scientific exploration of the enormous pool of synthetic, biological, and natural products.\(^2\) As the conventional cancer therapies failed to fulfill the criteria for a successful cancer therapy, recent research revolves around the urgency to develop suitable chemotherapy for the treatment of cancer with no toxic effects.\(^3\) It was reported that cancer therapies utilizing the natural products like plants is a relatively new but very promising strategy in preventing cancer.\(^4\) Plant-derived natural products such as flavonoids, alkaloids and terpenes have received significant attention in the recent years due to

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their diverse pharmacological properties including cytotoxic and cancer chemo preventive effects. In light of the high need for effective anticancer agents, and the association of fruit and vegetable consumption with reduced cancer risk, edible plants are increasingly being considered as sources of anticancer drugs and is regarded as one of the most visible fields for cancer control.

Nature is an attractive source of new therapeutic candidate compounds as a huge chemical diversity is found in millions of species of plants, animals, marine organisms, and microorganisms as potential anticancer agents. A large number of plant-derived compounds have been identified for prevention and treatment of cancer, such as paclitaxel, vinblastine, camptothecin, resveratrol, melatonin, sulforaphane, genistein, brassinin, and lycopene.

Fruit peels are the protective coverings of the fruits which are generally considered as a waste material and are discarded after fruits consumption but many recent investigations have proved that these fruit peels may contain many bioactive constituents which have various significant medicinal and pharmacological properties. On the basis of this background, we aimed to investigate the in vitro anticancer potential of five Indian fruit peels through 3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on HepG2 cells since no such work has been carried out so far.

Materials and Methods

Selection of Fruits

The traditional fruits were collected from local market of Rohtak, Haryana (India). Five fruits were selected on the basis of various ethnopharmacological reports and investigations as anticancer potential or similar activities of these fruits. The selected fruits are mentioned below in Table 1.

Preparation of Extracts

100 g of dried, powdered fruit peels of each fruit were subjected for maceration for 7 days using ethanol: water (80:20) as a solvent. The mixture was shaken at regular intervals. The resultant extracts were then filtered and concentrated to remove the solvent and dried completely by lyophilisation. The dried extracts were suspended in water for the cytotoxic assay.

Cell Lines Used

Human HepG2 hepatocellular carcinoma (HCC) cell lines were procured from National Centre for Cell Science, Department of Biotechnology, Government of India, Pune, Maharashtra, India.

In vitro Assay for Cytotoxic Activity 3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide Assay

The cytotoxicity of all the extracts was determined by a tetrazolium (MTT) assay. HepG2 cells were plated (Bioklenz laminar air flow) onto 96 well plates on a cell density of 2 × 10^4/ml per well in 100 µl of RPMI 1640 and allowed to grow in a CO₂ incubator (New Brunswick Galaxy 170S) for 24 h (37°C, 5% CO₂). The medium was then replaced by fresh medium containing different concentrations of the sample (5, 10, 20, 40, and 80 µg/ml) for further 48 h incubation. Then 20 µl MTT stock solution (5 mg/ml in phosphate-buffered solution) was added to each well and incubated for 5 h. The medium was removed, and 200 µl DMSO was added to each well to dissolve the MTT metabolic product. The absorbance was measured at 560 nm by ELISA reader. Percentage viability of samples was calculated using the formula:

\[
\% \text{ cytoviability} = \frac{A_{560} \text{ of treated cells} - A_{560} \text{ of control cells}}{A_{560} \text{ of control cells}} \times 100\%.
\]

The percentage viability was plotted against concentration and linear regression curve were established in order to calculate inhibitory concentration 50 (IC₅₀) values of the extracts in order to determine their efficacy. IC₅₀ value represents the concentration of a sample (µg/ml) necessary to inhibit 50% of cells.

Fractionation and Further Analysis

Extract with least IC₅₀ value was fractionated with petroleum ether, ethyl acetate, and chloroform to obtain the respective fractions. The aqueous fraction was obtained as the remaining fraction. These fractions were suspended in water; and MTT assay was carried out. The preliminary phytochemical screening was carried out by chemical tests to determine the presence/absence of major phytoconstituents. Total flavonoid content was estimated using standard method as rutin equivalent.

Results

Extraction

The selected fruit peels for the present study were extracted and fractionated as detailed. The extractive value of each fruit peel extract is presented in Table 2.

3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide Assay for Peel Extracts

MTT assay was carried out for each extract against HepG2 cells. The regression graphs plotted for calculation of IC₅₀ values are represented in Figures 1 and 2. The results reveal that out of observed IC₅₀ values of all extracts, papaya has shown best activity with least IC₅₀ value of 18.5 µg/ml [Table 3]. Further, the fractionation of papaya peel extract was carried out with petroleum ether, ethyl acetate, and chloroform successively and the remaining fraction was considered as an aqueous fraction.

Table 1:

| Common name | Biological name with family | Part used |
|-------------|-----------------------------|-----------|
| Banana      | Musa acuminata (Musaceae)   | Peels     |
| Guava       | Psidium guajava (Myrtaceae) | Peels     |
| Lemon       | Citrus limon (Rutaceae)     | Peels     |
| Orange      | Citrus sinensis (Rutaceae)  | Peels     |
| Papaya      | Carica papaya (Caricaceae)  | Peels     |

Table 2:

| Common name | Percentage of yield of extract (% w/w) |
|-------------|----------------------------------------|
| Banana      | 10.73                                  |
| Guava       | 9.98                                   |
| Lemon       | 6.48                                   |
| Orange      | 13.93                                  |
| Papaya      | 12.68                                  |
3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide Assay for Fractions of Papaya Peel Extract

The fractionation of the papaya peel extract was carried out with petroleum ether, ethyl acetate, and chloroform successively and the remaining fraction was considered as an aqueous fraction. The MTT assay as described above was again performed on four fractions of papaya fruit peel extract and IC$_{50}$ [Table 4] values were determined. The regression graphs plotted for calculation of IC$_{50}$ values are represented in Figure 3.

Phytochemical Screening of Papaya Peels Extract

Preliminary phytochemical analysis

Preliminary phytochemical analysis of papaya peel extracts showed the presence of carbohydrates, proteins, flavonoids, which are enlisted in Table 5. The value for total flavonoid content is depicted in Table 6.

Discussion

The last two decades have witnessed a clear global inclination for drugs and health foods from natural resources. With the realization of health hazards and toxicity associated with the indiscriminate use of synthetic drugs and remedies, introduction of postmarketing surveillance and active adverse drug reaction monitoring, more than ever a need has been felt for an integrated system of medicine which would minister to the human body as a whole, safety being a major concern. The solution may perhaps be hidden into the traditional system of medicines such as “Ayurveda” or the “Science of life.” Fruit peels considered as waste part of the total fruit, contain many bioactive constituents which are responsible for several potential pharmacological and medicinal properties. Scientific studies conducted in the recent past have concluded that fruits and fruit peels rich in phenolics and flavonoids have shown significant antioxidant and anticancer activities.[9,11] Many fruits such as guava, banana, papaya, orange, lemon, apple, litchi possess proven medicinal activities as whole fruit, seeds, leaves, and as peels[12-14] and many of them are reported to have anticancer potential such as lemon,[15] orange,[16] papaya,[17] guava.[18] On the basis of this, a step was taken forward to evaluate the anticancer potential of five Indian fruit peels. Since cultured cancer cells are valuable reagents for rapid screening of potential anticancer agents as well as for elucidating the mechanism of their activity so this procedure was used in the present study.

HCC is one of the most common malignancies, responsible for an estimated one million deaths annually.[19] Therefore, it becomes important to discover new agents which are effective on growth inhibition of HCC. Taking this into consideration, the present study was conducted on HepG2 cell lines. HepG2 are adherent, epithelial-like cells growing as monolayers and in small aggregates, have a model chromosome number of 55. HepG2 cell line was derived from the liver tissues. These HepG2 cell lines have previously been used for evaluation of the cytotoxic potential of fruit extracts.[20] As in the present study, five fruit peel extracts, i.e., banana, guava, orange, lemon, and papaya were assessed for the anticancer activity against HepG2 liver cancer cells. The hydroalcoholic extracts of the fruit peels were prepared by maceration for 7 days in ethanol: water (80:20). The anticancer activity was carried out by MTT assay. The extracts of banana, guava, orange and papaya showed good anticancer activity with IC$_{50}$ values 31.7, 27, 95.5, and 18.5 µg/ml, respectively. Among these papaya extract exhibited the best anticancer activity as
Evidence from IC_{50} values. Various fruit extracts have already been previously reported to have anticancer properties such as cranberry, lemon, apple, strawberry, red grape, banana, and grapefruit that showed potent antiproliferative activity toward the HepG2 cell line.\cite{21} Rowanberry, raspberry, lingonberry, cloudberry, arctic bramble, and strawberry towards HeLa (human cervical cancer)\cite{22}. The Lemon peel and Orange peel extract did not show significant anticancer activity. The Lemon peel and orange peel extracts do not possess significant anti-tumor properties as evident from previously reported study.\cite{23,24} The papaya peel extract was selected for further study. Various fractions (petroleum ether, ethyl acetate, chloroform, and water) of the papaya peel extract were prepared by liquid-liquid partitioning, and these fractions were then subjected to the anticancer study by MTT assay against the HepG2 cell lines. The IC_{50} values of various fractions were: Ethyl acetate - 18.0 µg/ml, chloroform - 25.0 µg/ml, and aqueous fraction - 17.3 µg/ml. Petroleum ether fraction did not reveal any significant results.

Though MTT is not the only sure and certain method for evaluation of anticancer activities, it is only a step to check the cytotoxicity of the sample toward cancer cell lines and hints that the sample may have the anticancer activity which can be confirmed by other assays such as XTT, trypan blue dye exclusion followed by in vivo studies and clinical trials which in this regard is also worth investigation.\cite{17,25} Papaya has shown anticancer activities in some previously conducted study;\cite{26} moreover, several studies have proved that flavonoids are responsible for the anticancer properties present in many fruits.\cite{19,26} Based on this, it can be said that fruit may peels may possess anticancer potential against HCC, particularly papaya fruit peels.

**Conclusion**

The aqueous papaya peel extract conferred noticeable cytotoxicity against the prostate and HepG2 cancer cell lines supporting toward its development as a potential therapeutic agent for the treatment of cancer with further insight toward the possible mechanisms of actions. This is the first report on the cytotoxic potential of the peels of Carica papaya, and the results obtained strongly provide support for the ethnobotanical use of the fruit.

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Nil.

**Conflicts of Interest**

There are no conflicts of interest.

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