PRELIMINARY PHYTOCHEMICAL ANALYSIS AND CYTOTOXICITY POTENTIAL OF PINEAPPLE EXTRACT ON ORAL CANCER CELL LINES

ANIRUDH MENON1*, VISHNU PRIYA V2, GAYATHRI R2

1Department of Biochemistry, Saveetha Dental College, Saveetha University, Chennai, Tamil Nadu, India. 2Department of Biochemistry, Saveetha Dental College, Saveetha University, Chennai, Tamil Nadu, India. Email: aniruddhmenon@icloud.com

Received: 07 June 2016, Revised and Accepted: 22 June 2016

ABSTRACT

Objective: This study aims at performing a preliminary phytochemical analysis to evaluate the phytochemical composition of pineapple extract and its cytotoxicity potential on oral cancer cell lines.

Methods: Preliminary phytochemical analysis of pineapple extract was done, 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide assay for evaluating the cytotoxicity potential of the extract on oral cancer cell lines was performed.

Results: Phytoconstituents such as flavonoids, coumarins, and phenols were present in the pineapple extract. The extract also exhibited increased cytotoxicity with increased concentration.

Conclusion: This study is conducted to see if pineapple extract is effective in treating oral cancer in a natural way instead of harmful treatments.

Keywords: Cytotoxicity, Pineapple extract, Anticancer drug.

INTRODUCTION

Pineapples have a long tradition as a medicinal plant among the natives of South and Central America. The first isolation of bromelain was recorded by the Venezuelan chemist Vicente Marcano, in 1891, by fermenting the fruit of pineapple. In 1892, Russell Henry Chittenden, assisted by Elliott P. Joslin and Frank Sherman Meara, investigated the matter more completely, and called it “bromelin.” Later, the term “bromelain” was introduced and originally applied to any protease from any member of the plant family Bromeliaceae.

Bromelain is present in all parts of the pineapple plant, but the stem is the most common commercial source [citation needed], presumably because large quantities are readily available after the fruit has been harvested. A concentrate of proteolytic enzymes enriched in bromelain is approved in Europe for the debridement (removal of dead tissue) of severe burn wounds under the trade name NexoBrid. Bromelain has not been scientifically proven to be effective in treating any other diseases, and it has not been licensed by the Food and Drug Administration for the treatment of any disorder. Available in some countries as a product under the name Ananase, bromelain gained its reputation for various uses in folk medicine. As a potential anti-inflammatory agent, it may be useful for treating arthritis but has neither been confirmed in human studies for this use nor is it approved with a health claim for such an effect by the Food and Drug Administration or European Food Safety Authority.

While there have been studies which positively correlated the use of bromelain with reduction of symptom severity in osteoarthritis, the majority of the studies have methodological issues that make it difficult to draw definite conclusions, as none definitively established efficacy, recommended dosage, long-term safety, or adverse interaction with other medications. Systemic enzyme therapy (consisting of combinations of proteolytic enzymes such as bromelain, trypsin, chymotrypsin, and papain) has been investigated in Europe to evaluate the efficacy of proteolytic enzymes in the treatment of breast, colorectal, and plasmacytoma cancer patients. Bromelain supplements, when taken with other medications (amoxicillin, antibiotics, anticoagulant/antiplatelet drugs), may increase the risk associated with heart rate, blood clotting, and bleeding after surgery.

Cytotoxicity

Cytotoxicity is the quality of being toxic to cells. Examples of toxic agents are an immune cell or some types of venom, e.g., the Black Widow spider or The King Cobra.

Cytotoxicity assays are widely used by the pharmaceutical industry to screen for cytotoxicity in compound libraries. Researchers can either look for cytotoxic compounds if they are interested in developing a therapeutic that targets rapidly dividing cancer cells, for instance; or they can screen “hits” from initial high-throughput drug screens for unwanted cytotoxic effects before investing in their development as a pharmaceutical. Assessing cell membrane integrity is one of the most common ways to measure cell viability and cytotoxic effects. Compounds that have cytotoxic effects often compromise cell membrane integrity. Vital dyes, such as trypan blue or propidium iodide, are normally excluded from the inside of healthy cells; however, if the cell membrane has been compromised, they freely cross the membrane and stain intracellular components [1]. Alternatively, membrane integrity can be assessed by monitoring the passage of substances that are normally sequestered inside cells to the outside. One molecule, lactate dehydrogenase (LDH), is commonly measured using LDH assay. LDH reduces nicotinamide adenine dinucleotide (NAD) to NAD hydrogen which elicits a color change by interaction with a specific probe [2]. Protease biomarkers have been identified that allow researchers to measure relative numbers of live and dead cells within the same cell population. The live-cell protease is only active in cells that have a healthy cell membrane and loses activity once the cell is compromised, and the protease is exposed to the external environment. The dead cell protease cannot cross the cell membrane and can only be measured in culture media after cells have lost their membrane integrity [3].

Cytotoxicity can also be monitored using the 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) or with 2,3-bis-(2-methoxy-4-nitro-5-sulfo phenyl)-2H-tetrazolium-5-carboxanilide (XTT), which yields a water-soluble product, or the MTS assay. This assay measures the reducing potential of the cell using a colorimetric reaction. Viable cells will reduce the MTS reagent to a colored formazan product. A similar redox-based assay has also been developed using the fluorescent dye, resazurin. In addition to using dyes to indicate...
To 1 ml of plant extract, 2 ml of 5% ferric chloride was added. Formation of purple or bluish-brown ring indicates the presence of phytosteroids [16].

Test for quinones
To 2 ml of plant extract, 3 ml of chloroform and 1% ammonia solution was added. Formation of pink indicates the presence of quinones [14].

Test for glycosides
To 2 ml of plant extract, 3 ml of chloroform and 1% ammonia solution was added. Formation of pink indicates the presence of glycosides [15].

Test for cardiac glycosides
To 0.5 ml of extract, 2 ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was underlayered with 1 ml of concentrated sulfuric acid. Formation of brown ring at the interface indicates the presence of cardiac glycosides [12].

Test for terpenoids
To 0.5 ml of extract, 2 ml of chloroform was added and concentrated sulfuric acid was added carefully. Formation of red-brown at the interface indicates the presence of terpenoids [12].

Test for phenols
To 1 ml of the extract, a few drops of phenol Gocalite reagent were added followed by few drops of 15% sodium carbonate solution. Formation of blue or green color indicates the presence of phenols [14].

Test for coumarins
To 1 ml of extract, 1 ml of 10% NaOH was added. Formation of yellow indicates the presence of coumarins [14].

Steroids and phytosteroids
To 1 ml of plant extract, an equal volume of chloroform is added and subjected with few drops of the concentrated sulfuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish-brown ring indicates the presence of phytosteroids [16].

Phlobatannins
To 1 ml of plant extract, few drops of 2% HCL were added the appearance of red precipitate indicates the presence of phlobatannins [12].

Anthraquinones
To 1 ml of plant extract, few drops of 10% ammonia solution were added, appearance pink precipitate indicates the presence of anthraquinones [12].

MTT assay for cytotoxicity
The MTT assay (Mossman, 1983) is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. Cells were maintained in Dulbecco’s modified Eagle’s medium, supplemented with 10% fetal bovine serum, at 37°C in humidified atmosphere with 5% CO₂.
The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately $1.2 \times 10^4$ cells/well and allowed to attach overnight at 37°C. The medium was then discarded, and cells were incubated with different concentrations of the samples (25, 50, 75, 100, and 125 µg) for 24 hrs. After the incubation, medium was discarded, and 100 µl fresh medium was added with 10 µl of MTT (5 mg/ml). After 4 hrs, the medium was discarded, and 100 µl of dimethyl sulfoxide was added to dissolve the formazan crystals. Then, the absorbance was read at 570 nm in a microtiter plate reader. Cyclophosphamide was used as a positive control (PC) [17].

Cell survival was calculated by the following formula:

Viability % = (Test OD/Control OD) × 100

Cytotoxicity % = 100 − Viability %

RESULTS

Table 1 and Fig. 1 shows the phytochemical analysis.

Table 2 shows the percentage of cell viability of sample and PC against KB cells.

Graph 1 shows the effect of cell viability of sample and PC in KB cells.

Table 3 shows the percentage of cytotoxicity of sample and PC against KB cells.

Graph 2 shows the effect of cytotoxicity of sample and PC against KB cells.

DISCUSSION

The main constituents found in the extract were flavanoids, phenols, coumarins, steroids, terpenoids, and quinones. Secondary metabolites such as alkaloids, quinones, and phenols, present in pineapple extract showed anticancer potential. The presence of phenols suggests the antioxidant activity of the extract. Tannins which are a group of phenolic compounds that are known antimutagenic property and can act against cancer cells. Cytotoxicity analysis using varying concentrations of pineapple extract was done. As shown in Tables 1 and 2, the viability of the KB cell lines shows a gradual change as the concentration of the extract is increased. Pineapple extract exhibited increasing cytotoxicity.
with increasing concentration. This is also evident from the graphical representations (Graphs 1 and 2).

CONCLUSION

Natural products are used widely nowadays to avoid the various side effects caused by carcinogenic drugs. The phytoconstituents found in the extract reveals the antioxidant property of the extract. The study exposed the cytotoxic potential and antitumor properties of pineapple extract. The potential to develop pineapple extract as an anticancer drug is a thrust area for future research in drug designing industry.

REFERENCES


Author Queries:?

AQ3: Kindly provide reference citation
AQ4: Kindly review the sentence.
AQ5: Kindly provide complete reference details
AQ6: Kindly cite reference 19 in text part and also cite all references in chronological order
AQ7: Kindly provide Tables 1 and 3 captions
AQ8: Kindly provide figure 1 captions
AQ9: Kindly provide Graphs 1 and 2 captions