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In Vitro Evaluation Of Cytotoxicity and Antioxidant Efficacy Of Bromelain

Research Article

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Abstract

Introduction: There is a growing interest to sustainably explore the potent antioxidant capacity of plant species. Pineapple has been incorporated in various herbal preparations since ancient times. Bromelain, a complex mixture of protease found in pineapples is widely administered for its clinical and therapeutic applications.
The present study was aimed to assess the cytotoxicity and antioxidant activity of bromelain.
Materials and Methods: Cytotoxic effect by Brine shrimp lethality assay, Antioxidant effect by DPPH assay at 10µl, 20µl, 30µl, 40µl and 50µl.
Results: Bromelain exhibits least cytotoxicity at 10µl, 40µl and 50µl where as moderate cytotoxicity at 20µl and 30µl. The antioxidant property of bromelain increases with an increase in the concentration. The percentage of inhibition was 38.7% at 10µl, 54.8% at 20µl, 60.5% at 30µl, 64.2% at 40µl and 79.9% at 50µl.
Conclusion: Bromelain extracts are predominant sources of natural antioxidants with least cytotoxicity and can serve as an alternative approach to oxidative stress management.

Keywords: Bromelain; Brine Shrimp Lethality Assay; Antioxidant Activity; Cytotoxicity.

Introduction

Free radicals, a.k.a Reactive Oxygen Species are unstable molecules generated by all cells in day to day biological processes [1]. However, uncontrolled production of these molecules can have a deleterious impact on cellular operations like cell membrane fluidity, permeability and ion channels activity [2]. The major threatening factor is that these free radicals form the basis for DNA damage causing mutagenic changes and cellular death.

It is very well known that oxidative stress is caused by free radicals and their derivatives [3]. This is majorly responsible for disparity in redox homeostasis [4]. It is also one of the prime factors leading to the development of chronic disorders, diabetes mellitus, coronary heart ailment, ageing and cancer.

Plants are a potent source of antioxidants [5]. There is a wide growing interest in natural antioxidants present in plants that might help to curb down the oxidative damage. Essential oils, flavonoids and phenolic compounds are the various bioactive components present in plants that are known to contribute to their antioxidant activity.

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Pineapple has been a part of traditional folk medicine since ancient times. It persists to be an essential component in various herbal preparations [6].

It belongs to the family Bromeliaceae. It is predominantly grown in Thailand, Indonesia, Malaysia, China and India. It has been used as a medicinal plant and these medicinal qualities are attributed to bromelain (EC 3.4.22.32) [7].

Bromelain is a complex natural mixture of proteolytic enzymes and possesses remarkable therapeutic properties. Bromelain has not only been used to treat various health problems, it is also a popular nutritional supplement to improve health. It promotes bioavailability and reduces the side effects associated with various antibiotics. Further more, it acts as an immunomodulator and comprises notable anti-edematous anti-thrombotic and anti-inflammatory properties [6].

About 80% of the world's population are dependent on plant based medicines for their fundamental health needs [8]. However, majority of plants have not yet encountered comprehensive studies to identify their bioactive compounds [9].

In this study, the antioxidant and cytotoxicity of bromelain is determined to find out their suitability in therapeutic and biochemical applications.

Materials and Methods

This in vitro study was conducted in the Nanomedicine Lab, Saveetha Dental College, Chennai.

Cytotoxic Effects

Brine Shrimp Lethality Assay: It is a general bioassay that is capable of detecting a broad spectrum of bioactivity in crude extracts (Fig. 1). The commercial availability of cost efficient brine shrimp eggs, the safety and the ease of handling the assay, as well as no exceptional technological specifications makes this an efficient tool for the phytochemistry laboratory. This assay has been predominantly used to biomonitor the isolation of plant cytotoxic compounds [10].

Preparation Of Bromelain Extracts

Bromelain powder was obtained from online retailers of South India. Bromelain extract was formulated by adding 0.5mg of bromelain powder to 5ml of distilled water. The soluion was then filtered using What man filter paper and the clear extract was then transferred to an airtight container.

Hatching The Shrimp

The brine shrimp eggs were hatched in artificial sea water prepared through dissolving 0.5 g of sea salt in 150ml of distilled water (Fig. 2). After 24 hours of incubation at room temperature (27-29 degree celsius), the larvae (nauplii) were attracted to one side of the artemia tank with a light source and collected with pipette (Fig. 2).

6 tubes were taken that were filled with artificial sea water. 10 nauplli's were added to each of the tubes. Bromelain was loaded in the concentration range of 10µl, 20µl, 30µl, 40µl, 50µl.

A control tube was also prepared by adding 3ml of artificial sea water and 10 nauplii's. The tubes were kept for 24 hours incubation. After the incubation, the tubes were observed using a magnifying glass. The live and dead nauplii's were counted and percentage death was calculated.

Percentage death= Number of dead nauplii X 100/Number of dead nauplii-Number of live nauplii

Antioxidant Activity

DPPH Radical Scavenging Assay was executed to monitor the antioxidant potential of plant crude extract. DPPH is a lipophilic

Figure 1. Cytotoxic activity by Brine Shrimp Lethality Assay.

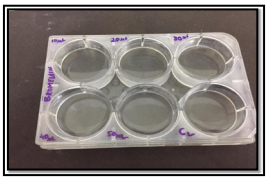


Figure 2. Live nauplii eggs collected in a petri dish.

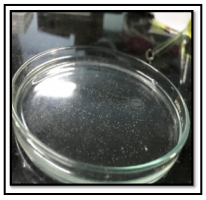
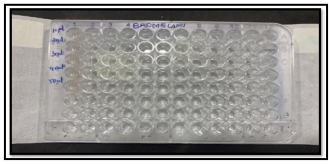


Figure 3. Antioxidant activity by DPPH Radical Scavenging assay.



free radical, nitrogen centered with purple colour (Fig 3). The antioxidant donates an electron to DPPH radical there by colour changes to pale yellow gradually.

Test group: 10µl, 20µl, 30µl, 40µl, 50µl was taken in 5 test tubes respectively. To each tube,1ml of DPPH (2,2-diphenyl-1-picryl-hydrazyl) was added. 1990µl, 1980µl, 1970µl, 1960µl, 1950µl of 50% methanol solution was added to the test tube containing 10ul, 20ul, 30ul, 40ul, 50ul of bromelain respectively.

Control group: 1ml of DPPH was added to 2ml of methanol solution. Standard group: Ascorbic acid was used as standard.

The test tubes were incubated in a dark cupboard for around 20 minutes. Absorbance was measured at 517nm in UV Spectrophotometer.

% inhibition was calculated using following formula:

% of inhibition=Control Absorbance-Sample Absorbance X 100/Control Absorbance

Results

Table 1 depicts the cytotoxicity of bromelain extract. The results show that there is 10% death of nauplii eggs at 10µl, 40µl and 50µl respectively. Hence the study affirms that bromelain exhibits least cytotoxicity at 10µl, 40µl and 50µl where as moderate cytotoxicity at 20µl and 30µl. There was no significant increase in percentage of death with an increase in the concentration. The maximum percentage of death was 20% noted at both 20µl and 30µl respectively.

The involvement of free radicals, especially their increased production, appears to be a feature of most of human diseases including cardiovascular diseases and cancer. In this regard, the antioxidant activity of synthesized bromelain extract was assessed by DPPH scavenging assays. The DPPH free radical is stabilized when it accepts electrons. The DPPH radical is purple in colour with a maximum absorbance at 517 nm. The observed results in the DPPH assay show free radical inhibition by bromelain.

Fig.4. signifies the antioxidant activity of bromelain extract. The values of antioxidant property of bromelain are lower than the standard values at all concentrations. However, the antioxidant property of bromelain increases with an increase in the concentration. The percentage of inhibition was 38.7% at 10µl, 54.8% at 20µl, 60.5% at 30µl, 64.2% at 40µl and 79.9% at 50µl.

Discussion

The World Health Organisation(WHO) signifies that majority of the world's population do not have access to adequate health care services. This is due to the fact that poor people neither have access to nor can afford present health care services. However medicinal plants offer alternative remedies with tremendous opportunities. They not only provide access and affordable medicine to poor people; they can also generate income, employment and foreign exchange for developing countries. Excessive production of free radicals can have a deteriorating effect on cell membranes cell organelles. These radicals are the key factors leading to mutagenic changes and cellular death [11]. Plants are the primary source of natural antioxidants. This antioxidant activity of plants is predominantly associated with flavanoids, isoflavanoids, phenolic and acanthocyanins content [12].

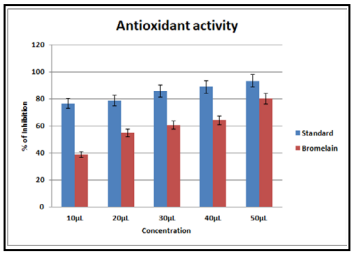
Bromelain is a complex mixture of proteolytic enzymes. Its therapeutic value may be attributed to glycoprotein, the potent ingredient in bromelain [13]. Recent studies have stated that bromelain has the capacity to alter the crucial pathways that stimulates malignancy. In a study conducted by Beez et al, chemically induced mouse skin papillomas were treated with bromelain and they noticed a significant decrease in tumour formation and tumour volume.

In the current study, the values of antioxidant property of bromelain are lower than the standard values at all concentrations. However, the antioxidant property of bromelain increases with an increase in the concentration. The percentage of inhibition was 38.7% at 10µl, 54.8% at 20µl, 60.5% at 30µl, 64.2% at 40µl and 79.9% at 50µl Further more, there is 10% death of nauplii eggs at 10µl, 40µl and 50µl respectively. Hence the study affirms that bromelain exhibits least cytotoxicity at 10µl, 40µl and 50µl where as moderate cytotoxicity at 20µl and 30µl. There was no significant increase in percentage of death with an increase in the concentration. The maximum percentage of death was 20% noted at

Table 1. Cytotoxicity of bromelain extract.

Conc in µl	10µl	20µl	30µl	40µl	50µl	control
Viable nauplii	9	8	8	9	9	10
Percentage of death	10%	20%	20%	10%	10%	0%

Figure 4. Antioxidant property of Bromelain extract.



both 20µl and 30µl respectively. Therefore, we can indicate that bromelain exhibits least cytotoxicity and improved antioxidant potential at its higher concentrations.

Based on the findings of the current study, we can state that bromelain extract can be used as an alternative to commercially available antioxidant agents.

Conclusion

The results of the present study indicated that bromelain extracts are a potential source of natural antioxidants and suggests that bromelain might be explored as a viable source of potent antioxidants from oxidation. Further investigations are necessary for the chemical characterization of the active compounds and more comprehensive biological assays.

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