Research Article

Comparative *in-vivo* Free Radical Scavenging Activity of Pineapple and *Eclipta alba* Extracts by NO Assay.

Geethika B*, Gayathri R, Vishnu Priya V
Department of Biochemistry, Saveetha Dental College, Chennai, Tamil Nadu, India.
***Corresponding author's E-mail:** drgeethikababu@gmail.com

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ABSTRACT

The aim of study is to compare the free radical scavenging activity of pineapple extract and eclipta alba extract by NO assay. Free radical scavenging is associated with the antioxidant potential of a compound. Antioxidant shields our body from cancer. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Pineapple is a tropical plant with edible multiple fruit and the most economical significant plant in the Bromeliaceae family. Eclipta Alba is a herb that has traditionally been used in Ayurvedic medicine for being a liver tonic and having beneficial effects on diabetes, eye health and hair growth. The reason of the project is to find out and compare the antioxidant effect of pineapple extract and eclipta alba extracts. Extracts with high antioxidant activity can be used in anti cancer formulations.

Keywords: Antioxidant activity, Nitric oxide scavenging, Pineapple extract, Eclipta alba.

INTRODUCTION

Organic compounds called secondary metabolites which helps in various functions are produced in diverse array by plants. Terpenes, phenolics and N and S containing compounds are the three major groups of metabolites.[1]

The beneficial effects of natural products have made humans use them ever since.

The field of organic chemistry is influenced by the discovery of new bioactive natural products as demonstrated by the recent models of drugs like epothilon which is anti-cancerous in nature, rapamycin which is an immunosuppressant and quite a few drugs.[2]

The antioxidant activity and free radical scavenging ability of phenolic compounds have made the phenolic compounds gain much attention in recent years.

Phenolic compounds are widely distributed in plants and possess potential beneficial implications in human health.[3] Antioxidants reduce rancidity, decelerate the formation of toxic oxidation products, sustain nutritional quality, and increase time period, when added to foods.[4]

These antioxidants may aid to relieve oxidative stress, intercepting free radicals from harming biomolecules such as proteins, DNA, and lipids.[5]

Free radical can be defined as any molecular species that has the ability of independent existence and that which has an unpaired electron in an atomic orbital.

Free radicals share certain properties with most radicals which is due to the presence of an unpaired electron.

Many radicals are unstable and highly reactive. They can behave as oxidants or reductants by either donating an electron to or accepting an electron from other molecules.[6]

Pineapple is commonly known as *Ananas comosus* L. belongs to the family *Bromeliaceae* is a tropical to subtropical fruit native to Thailand, Phillipines, China, Brazil and India.[7]

Pineapple has a high of citric and malic acid contents; citric acid concentrations in some cultivars exceed 8%.[8]

The fruit also possesses moderate amounts of ascorbic acid; 2 slices of pineapple contain ascorbic acid 100 mg.[9]

A component of the leaves which is steroidal in nature possesses oestrogen activity.[8]

Eclipta Alba (L.) commonly known as false daisy, is an annual herbaceous plant. There are three types of Eclipta Alba the white-flowering, the yellow-flowering, and the black-fruiting.

The three types of eclipta grow all over India by rivers, marshes, and lakes or on the foothills of the Himalayas.[10]

The antioxidant activity of the fruits are influenced by both the phenolic and ascorbic acid content of the fruits.

Phenolics can be divided into two groups by their antioxidant activity: primary and secondary antioxidants.

Chain reactions are terminated by primary antioxidants. Chain reactions could be terminated by mechanisms such as the donation of electron or hydrogen to the free radical, and the stable compound is formed.

Prevention of initiation of free radical chain reactions by methods such as metal chelation is bought about by secondary antioxidants.[11,12]

The objective of this study is to compare the nitro oxide radical scavenging activity of pineapple extract and eclipta alba.

MATERIALS AND METHODS

Materials

TPVG

Trypsin, Phosphate buffer saline, versene, glucose solution (TPVG) was prepared according to the manufacturer's protocol (HiMedia, India).

Foetal Bovine Serum

FBS contains the major growth factors required for the growth and survival of the cells.

A bottle of FBS was thawed, overnight at 2-8°C and allowed to equilibrate.

Incubated for 30 minutes at 56°C in a water bath, and is mixed for every five minutes.

Single use aliquots may be stored at 2-8°C for up to one month or at a nominal temperature of -20°C indefinitely.

Phosphate Buffer Saline

Sodium Chloride - 8g

Potassium chloride - 0.2g

Disodium hydrogen phosphate - 2.88g

Potassium dihydrogen phosphate - 0.2g

pH - 7.4

It was weighed, dissolved in 1000 ml sterile distilled water. It was filtered through Whatmann paper and autoclaved at 15 lbs for 15 minutes.

Sodium Bicarbonate

Sodium bicarbonate produces carbon dioxide which acts as buffering agent. It maintains suitable growth condition in the medium without changing the medium to alkaline.

Sodium bicarbonate was used at a concentration of 0.37% for DMEM and 0.2% for RPMI 1640.

L-Glutamine (3%)

Non autoclavable medium has L-Glutamine. It is needed for cell metabolism and replication.

It acts as growth promoter and supplement separately. L-Glutamine in the medium cannot be autoclaved as it gets denatured.

The media with Glutamine is sterilized by membrane filtration using 0.22-0.45 micron size filter.

Antibiotics

It is used to prevent contamination. Penicillin (100µg/ml) and Streptomycin (50µg/ml) are added as anti-bacterial agent that inhibits both gram positive and gram negative organisms.

Lipopolysaccharide 1 mg/mL (LPS, Stock)

1 mL of sterile PBS or cell culture medium is added per 1 mg of LPS and is vortexed. LPS is stored for daily use at -20°C. Repeated freezing/thawing should be avoided.

Greiss Reagent

1% Sulfanilamide

1% sulfanilamide was prepared by dissolving 1 gm of sulfanilamide in 100 ml of 2.5% phosphoric acid.

0.1% naphthylethylenediaminedihydrochloride

100 mg of naphthylethylenediaminedihydrochloride was dissolved in 100 mL of 2.5% phosphoric acid.

Both the solutions are stored in glass bottles at 4°C. Equal volumes of reagents A and B will be combined just prior to use to form the Griess reagent.

This solution should be used immediately after preparation and any remaining should be discarded.

Methods

Media Preparation

RPMI 1640 and DMEM media were prepared as per manufacturer's protocol (Hi Media, India) and filter sterilized.

The other components added to the media to prepare a complete media include FBS, Sodium bicarbonate and Antibiotics.

Maintenance of KB (ATCC CCL-17) Cell Line

Cell culture flasks were selected by observing under inverted microscope. For maintaining the KB cell line, sub culturing was done.

This was done to facilitate the cell growth by removing the cells from the medium and introducing them into a new fresh medium. Mostly, enzymatic methods were used for cell maintenance.

The enzyme used here was TPVG. Growth medium was removed from the flasks and the cells were incubated at 37°C after adding the enzyme. Initially all cells get detached from the surface.

The cells were suspended in 5ml of the medium.

The suspension was aspired few times to break cell clumps. Cell line, date of seeding and passage number was marked on the bottom of the T-flask.

5 ml of cell suspension was transferred to fresh T-Flasks.

Nitric Oxide Assay (NO Assay)

The nitric oxide assay was performed with slight modification.[13] After pre-incubation of KB cells (1.5 × 105 cells/mL) with LPS (1 µg/ml) for 24h, the extracts (50 µg, 100 µg, and 200 µg) were added and incubated for 48h. The quantity of nitrite in the culture medium was measured as an indicator of NO production. Amount of

nitrite, a stable metabolite of NO, was measured using Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediaminedihydrochloride in 2.5% phosphoric acid). Briefly, 100 µl of cell culture medium was mixed with 100 µl of Griess reagent. Subsequently, the mixture was incubated at room temperature for 10 min and the absorbance at 540 nm was measured in a microplate reader (Tecan, Switzerland). Fresh culture medium was used as a blank in every experiment.

Formula

The Inhibition activity was calculated using the formula:

$$\%\ of\ Inhibition = \frac{(A\ of\ control - A\ of\ Test)}{A\ of\ control} \times 100$$

RESULTS AND DISCUSSION

Nitric Oxide Scavenging Assay

Table 1: Nitric Oxide Scavenging Assay

Concentration (µg)	Control	Pineapple	Eclipta	Ascorbic acid	% Pineapple	% Eclipta	% of Ascorbic Acid
50	0.652	0.521	0.549	0.405	20.09202454	15.79754601	37.88343558
100	0.652	0.413	0.454	0.246	36.65644172	30.36809816	62.26993865
150	0.652	0.327	0.368	0.118	49.84662577	43.55828221	81.90184049

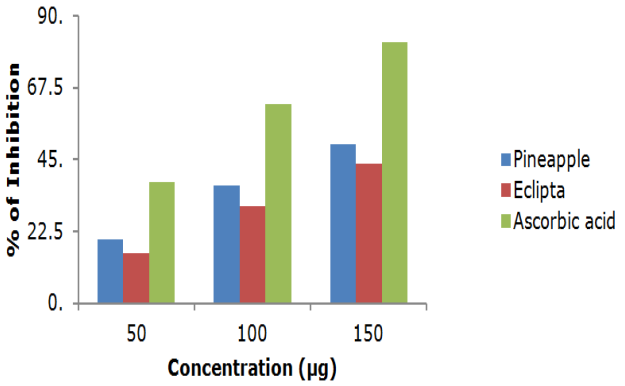

Figure 1

Nitric oxide (NO) is a potent inhibitor of physiological processes such as neuronal signaling, and inhibition of platelet aggregation, smooth muscle relaxation and regulation of cell mediated toxicity.[14]

Other than reactive oxygen species, nitric oxide is also used as an indicator in inflammation, cancer and other pathological conditions.[15,16] NO is known to be a omnipresent free-radical component, is supposed to have a indispensable role in neuromodulation or as a neurotransmitter in the CNS, which is distributed in tissues or organ systems.[17]

The nitric oxide radical scavenging activities of pineapple and eclipta alba extracts were shown in the Table 1, Figure 1 and are compared against the nitric oxide radical scavenging activity of ascorbic acid.

The percentage of inhibition of the pineapple extract showed 20.09 % in 50 µg, 36.65 % in 100 µg and in 49.85 % 150 µg. The percentage of inhibition of eclipta alba showed 15.79 % in 50 µg, 30.36 % in 100 µg, 43.55 % in 150 µg.

Hence the pineapple extract has better nitric oxide radical scavenging activity in comparison with eclipta alba to react with nitric oxide and thus the inhibition of generation of anions is achieved.

CONCLUSION

Antioxidant activity of both the sample were assessed using the NO assay. Both eclipta alba and pineapple extract show free radical scavenging activity. But, pineapple extract shows more antioxidant activity than eclipta alba in all the three concentrations - 50 µg, 100 µg and 150 µg.

The free radical scavenging activity of pineapple is suggested to be due to the presence of ascorbic acid in the fruit.[10]

Both the extracts could act as primary antioxidants but are weak secondary antioxidants. In future, both the extracts can be analyzed for anticancer activity. The promising antioxidant activity showed by the extracts suggest that drugs can be prepared using these extracts for the benefit of the society.

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