In Vitro Antioxidant And Anticancer Screening Of Giant Granadilla (Passiflora Quadrangularis).

Gopika. V. C [1], M. Vijey Aanandhi[2*]

*Department of Pharmaceutical chemistry and analysis, School of Pharmaceutical sciences, Vels Institute of Science, Technology & Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu-600117.

***Corresponding author: M. Vijey Aanandhi**

mvaanandhi@gmail.com

ABSTRACT

The present study was aimed to analyse the antioxidant and anticancer properties of hydro-alcoholic extract of leaf, fruit and seed of *Passiflora quadrangularis.* The collected plant parts were subjected to extraction with hydro-alcoholic solvent (Ethanol: water) in the ratio 1:1 by using Soxhlet apparatus. Invitro antioxidant study was performed using DPPH assay method by using ascorbic acid as standard. *Invitro* anticancer study was done using MTT assay in 2 cell lines: MCF-7 and HCT-116 using Doxrubicin as standard. Extraction yield was found to be more for leaf and least for seed, 36.05% and 4.13% respectively. DPPH assay shows similar antioxidant activity for selected parts ie., leaf, seed and fruit and the IC50 values found to be 63µg/mL, 68µg/mL and 60µg/mL respectively. For ascorbic acid the IC50 value was found to be 36.5µg/mL. For MTT assay performed in Human breast cancer cell line (MCF-7) the IC50 for standard drug Doxorubicin was found to be 4.54µg/mL and for leaf extract it is 81.40µg/mL. In case of Human colon tumor cell line (HCT-116) the IC50 value was found to be 4.98µg/mL for Doxorubicin and 93.04µg/mL for leaf extract. The tests shows that both fruit and seed extract show less anticancer activity in both cell lines selected when compared to leaf extract. The plant Passiflora quadrangularis is a good source of phytochemicals. Plant parts shows good antioxidant activity. Among the parts ie., leaf, fruits and seed, the hydro-alcoholic extract of leaf shows good anticancer activity for breast cancer and colon cancer cell lines.

Keywords: *Passiflora quadrangularis*, hydro-alcoholic extract, anticancer, antioxidant.

1. Research scholar, Department of Pharmaceutical chemistry and analysis School of Pharmaceutical sciences, Vels Institute of Sciences, Technology & Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu.

2*. Department of Pharmaceutical chemistry and analysis, School of Pharmaceutical sciences, Vels Institute of Science, Technology & Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu.

1.0 INTRODUCTION

According to estimates, more than 25% of the populations of United States will receive a cancer diagnosis at some point in their lives. Each year, more than 1.6 million new cases of cancer are discovered. The only treatments for less than a quarter of these patients will be surgery and/or local radiotherapy. The majority of those who are left will experience systemic chemotherapy at some point while they are unwell. Cancer chemotherapy aims to inflict a fatal cytotoxic event or apoptosis on cancer cells, which can halt the growth of a tumour.

The use of innovative drugs in addition to chemotherapy, hormone therapy, and gene-targeted therapy, as well as immune-targeted therapies, should be considered in the approach to cancer therapy. The development of pharmaceuticals and treatments is typically inspired by plants, and because plants have potent chemical components, many drugs and medicines are derived from plants. Herbs can be employed as a potential source of anticancer activity, however doing so requires medical care since many herbs might result in allergic reactions or digestive problems. Due to their ability to fight cancer, medicinal herbs are increasingly being used in conjunction with traditional medicines to treat cancer. In particular, the mechanisms of herb's activity against cancer can include cytotoxic effect, cancer cell proliferation inhibition, efficient reduction in tumor volume, and the ability to protect DNA from threatening radiation, thus increasing survival rates.[1-5]

The species *Passiflora quadrangularis* known with the common name Giant granadilla is mainly cultivated in many areas of the tropics, especially S. America, both for its edible fruit and also as an ornamental plant. This plant is promising for the search of bioactive compounds for the prevention and control of carcinogenic processes.[6]

2. MATERIALS AND METHODS

2.1 OBTAINING THE SAMPLES

Fresh fruits, leaves and seeds of the plant were collected from local areas of Thrissur, Kerala. The plant materials were taxonomically identified by senior scientist, Dr V B Sreekumar, Forest Botany Department, KFRI, Peechi, Thrissur.

2.2 EXTRACTION OF PLANT USING HYDRO-ALCOHOLIC SOLVENT

The materials were shade dried and coarsely powdered. The powdered material extracted with hydro-alcoholic solvent (water: ethanol, 50:50) by using sauxhlet apparatus. The crude extract

concentrated by using rotary evaporator. Thus produced extracts of leaf, fruit and seed of Passiflora quadrangularis were subjected to *in vitro* antioxidant and *in vitro* anticancer studies.

2.3 IN VITRO ANTIOXIDANT STUDY (DPPH ASSAY)

Preparation of the reagent: 4 mg of DPPH were dissolved in 100 ml of ethanol to create a 0.1 mM DPPH solution.

Working procedure: DMSO was used to prepare various quantities of sample extracts up to 40 l, and 2.96 ml of a 0.1 mM DPPH solution was then added. The reaction mixture was incubated at room temperature for 20 minutes in the dark. The mixture's absorbance was measured at 517 nm by a UV-Vis Spectrophotometer after 20 minutes. As a control, 3ml of DPPH was taken.

$$\% \text{ RSA} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where,
RSA is the Radical Scavenging Activity
Abs control is the absorbance of DPPH radical + ethanol
Abs sample is the absorbance of DPPH radical + sample extract.

2.4 IN VITRO ANTI-CANCER STUDY (MTT ASSAY)

The MTT assay's basic premise is that it measures cellular metabolic activity as a sign of cell viability, proliferation, and cytotoxicity. This colorimetric assay relies on the transformation of purple formazan crystals into a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, or MTT) by metabolically active cells. The MTT is converted to formazan by the NAD(P)H-dependent oxidoreductase enzymes found in the live cells. An ELISA plate reader is used to measure the absorbance at 570 nm after the insoluble formazan crystals have been dissolved using a solubilizing solution (100% DMSO)**.**

Cell lines and maintenance: The cell line / cell lines were procured from the National Centre for Cell Sciences (NCCS), Pune, India.

Cell culture media and maintenance: The cells were cultured in Dulbecco's Modified Eagles Medium (DMEM-Himedia), supplemented with 10% heat inactivated Fetal Bovine Serum (FBS) and 1% antibiotic cocktail containing Penicillin (100 U/ml), Streptomycin (100 µg/ml),

and Amphotericin B (2.5 µg/ml). The cell containing TC flasks (25 cm^2) were incubated at 37 ^{0} C at 5% CO_2 environment with humidity in a cell culture incubator (Galaxy® 170 Eppendorf, Germany).

Assay Procedure: On 96-well plates, the cells (2500 cells/well) were planted, and they were given 24 hours to adjust to the culture conditions of 37 °C and 5% CO2 in the incubator. The DMEM media (10 mg/mL) was used to create the test samples, and a 0.2 m Millipore syringe filter was used for filter sterilisation. Further diluted in DMEM media, the samples were added to the wells containing the grown cells at final concentrations of 6.25, 12.5, 25, 50, and 100 g/mL, respectively. Wells that hadn't been treated remained as the control. To reduce errors, each experiment was performed in three copies, and average results were used. The plates were then given another 24 hours of incubation after being treated with the test samples. The media from the wells were aspirated and thrown away after the incubation period. The wells received 100 L of a 0.5 mg/mL MTT solution in PBS. The plates were then left to generate formazan crystals for an additional 2 hours. After removing the supernatant, 100 L of 100% DMSO were added to each well. A microplate reader was used to measure the absorbance at 570 nm. Per plate, a blank consisted of two wells with no cells. Two cell lines (MCF-7 and HCT-116) were used in triplicate for each experiment. [7-17]

3. RESULTS AND DISCUSSION

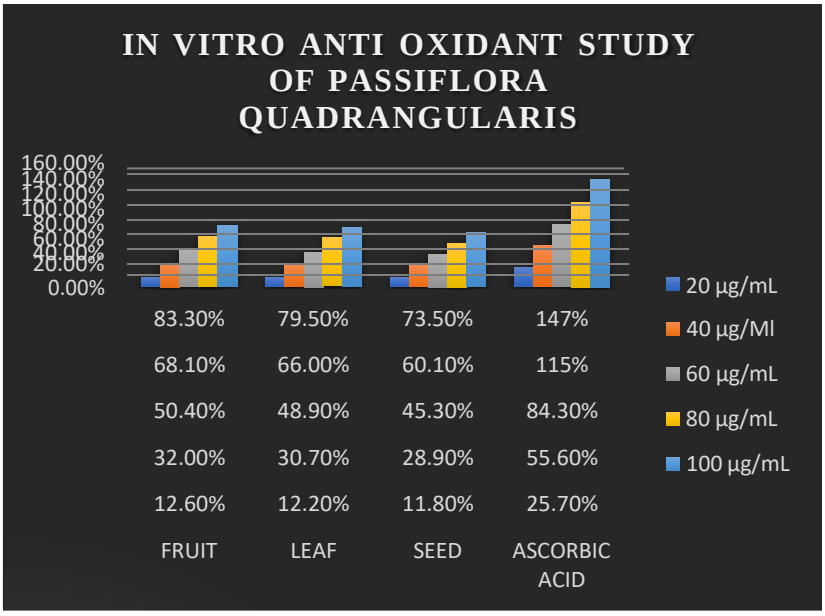

Figure 1: Free radical scavenging activity of *P. quadrangularis* extracts and ascorbic acid by DPPH radical inhibition.

Concentration (µg/ml)	Percentage viability
1	96.04
2	88.47
3	78.61
4	61.82
5	36.83
6.25	23.16
12.5	15.96
25	11.48
50	6.78
100	3.18
IC 50	**4.54**

Table 1: Percentage viability of different concentrations of Doxorubicin on MCF-7 cell line by MTT assay method

Concentration (µg/ml)	Percentage of viability
6.25	97.28
12.5	93.51
25	80.66
50	63.08
100	41.94
IC 50	**81.40**

Table 2: Percentage viability of different concentrations of Passiflora quadrangularis leaf extract on MCF-7 cell line by MTT assay method

Concentration (µg/ml)	Percentage of viability
6.25	98.57
12.5	95.86
25	89.04
50	84.12
100	75.46

Table 3: Percentage viability of different concentrations of Passiflora quadrangularis fruit extract on MCF-7 cell line by MTT assay method

Concentration (µg/ml)	Percentage of viability
6.25	98.20
12.5	94.52
25	81.31
50	68.28
100	53.82

Table 4: Percentage viability of different concentrations of Passiflora quadrangularis seed extract on MCF-7 cell line by MTT assay method

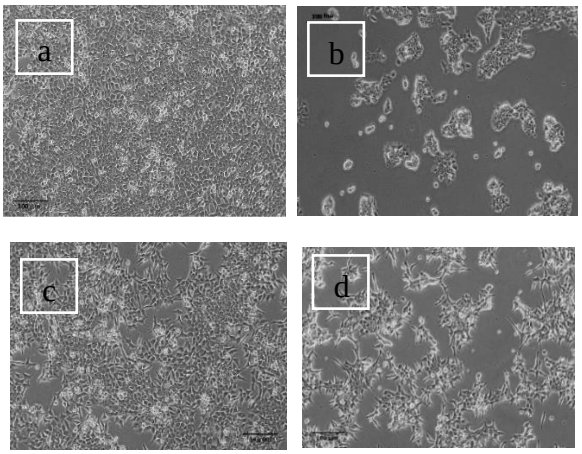

Figure 2: MTT assay cells (MCF-7) treated with a) Control cell b) *Passiflora quadrangularis* leaf extract c) *Passiflora quadrangularis* fruit extract d) *Passiflora quadrangularis* seed extract

Concentration µg/ml	Percentage viability
1	96.45
2	91.32
3	80.44
4	64.38
5	45.25
6.25	29.39
12.5	17.88
25	12.18
50	6.76
100	4.12
IC 50	**4.98**

Table 5: Percentage viability of different concentrations of Doxorubicin on HCT-116 cell line by MTT assay method

Concentration (µg/ml)	Percentage of viability
6.25	94.96
12.5	89.48
25	77.68
50	65.97
100	49.40
IC 50	**93.04**

Table 6: Percentage viability of different concentrations of Passiflora quadrangularis leaf extract on HCT-116 cell line by MTT assay method

Concentration (µg/ml)	Percentage of viability
6.25	99.38
12.5	96.11
25	93.06
50	89.31
100	82.10

Table 7: Percentage viability of different concentrations of Passiflora quadrangularis fruit extract on HCT-116 cell line by MTT assay method

Concentration (µg/ml)	Percentage of viability
6.25	98.63
12.5	91.12
25	83.96
50	70.97
100	62.53

Table 8: Percentage viability of different concentrations of Passiflora quadrangularis seed extract on HCT-116 cell line by MTT assay method

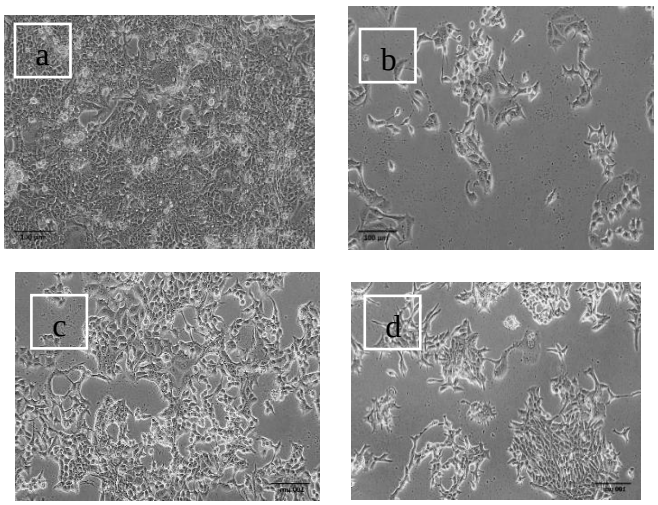

Figure 3: MTT assay cells (HCT-116) treated with a) Control cell b) *Passiflora quadrangularis* leaf extract c) *Passiflora quadrangularis* fruit extract d) *Passiflora quadrangularis* seed extract

This study is performed to compare the antioxidant and anticancer activities of *Passiflora quadrangularis* plant parts such as leaf, fruit and seed. Activities compared by performing in vitro studies. Antioxidant activity is determined by performing DPPH assay of the hydro-alcoholic extract of the plant parts. The extract was taken by using solvent, Ethanol: water (1:1) by Soxhlet extraction method. Extract was concentrated and used for the *in vitro* studies. In vitro anticancer studies performed by MTT assay method in 2 cell lines MCF-7 and HCT-116.

Extraction and results of the studies were compared for three parts of the plant ie., leaf, fruit and seed. The extraction yield was found to be more for leaf of the plant. For antioxidant studies by DPPH assay, it was found to be that antioxidant activity were similar for fruit, leaf and seed and IC50 values obtained were 60 µg/mL, 63 µg/mL and 68 µg/mL respectively. The activity was compared with standard drug Ascorbic caid. The IC50 value for Ascorbic was found to be 36.5 µg/mL.

For MTT assay the anticancer activities of the extracts were compared with that of standard drug Doxorubicin. For both cell lines selected for the study all extracts of the plant shows anticancer activity. Among the three extracts, leaf extracts show highest anticancer activity for MCF-7 and HCT-116. The IC50 values for leaf extract were found to be 81.40 µg/mL and 93.04 µg/mL for MCF-7 and HCT-116 respectively. The IC50 values for Doxorubicin was found to be 4.54 µg/mL and 4.98 µg/mL for MCF-7 and HCT-116 respectively.

4. CONCLUSION

The main aim of this study was to determine the antioxidant and anticancer activity of the extracts of plant *Passiflora quadrangularis* by in vitro method. The activities of extracts of leaf, fruit and seeds of the plant were compared. For in vitro antioxidant studies fruit extract showed highest activity and for in vitro anticancer studies leaf extract showed highest activity in both cell lines.

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