

Anti- Proliferative Activity Of Hylocereus Undatus Against Human Skin Melanoma (Skmel Cell Lines)

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ABSTRACT

Hylocereus undatus (Dragon fruit) is a tropical fruit of cactus species (Cactaceae) which has red peel with green fins. It contains rich antioxidants (vitamins and minerals that helps to prevent cancer, cardiovascular, diabetics, gastrointestinal, respiratory, and urinary disease), and low calorie dietary fibres. Every year, millions of people die from cancer, and most anticancer medications have a low specificity of action and significant toxicity, resultant in severe side effects. In the present research, the antioxidant and anticancer property of ethanol fruit pulp extract of Hylocereus undatus was studied and the result revealed that the ethanol fruit pulp extract of H. undatus showed 35.33% of inhibition at the 100 mcg/ ml concentration and the standard ascorbic acid showed 96.61% of inhibition at the 100 mcg/ ml concentration. The result represented that ethanol fruit pulp extract of H. undatus have moderate antioxidant activity when compared to the standard ascorbic acid. The L929 cell lines (murine fibroblast) were treated with ethanol fruit pulp extract of H. undatus. The percentage of cell viability of normal L929 cell lines and SKMEL cell lines (human skin melanoma) were treated with different concentrations of ethanol fruit pulp extract of H. undatus (6.25 µg/ml, 12.5 µg/ml, 50 µg/ml, 100 µg/ml) and their cytotoxic activity against Normal and Human skin melanoma was analysed. The results showed that higher the concentration of ethanol fruit pulp extract of H. undatus lower the viability of cell lines followed by higher the cytotoxic activity on cell lines. Dose dependant reduction in cell viability was observed in SK-MEL cells administered with different concentrations of the ethanol fruit pulp extract of H. undatus. The IC50 value of ethanol fruit pulp extract of H. undatus in SKMEL cell line was 74.32 µg/mL of the sample. The present research reported that the ethanol fruit pulp extract of H. undatus have a good anticancer property against the skin melanoma cell line thus, the fruit of H. undatus can be used as a natural remedy for cancer without any side effects..

Keywords: *Hylocereus undatus, antioxidant property, anticancer property, cytotoxic activity*

1. INTRODUCTION

White pitaya (Hylocereus undatus) has been widely marketed as an edible fruit crop (dragon fruit) and is being cultivated across tropical regions of the world (Le Bellec et al., 2006). White pitaya fruit is high in antioxidants, such as phenolics, flavonoids, and vitamins, such as ascorbic acid (Tang et al., 2021). The fruit pulp is also a rich natural source of betacyanins, which are known for their broad biological activities, including antioxidant and antibacterial activities, and seem to contribute to the fluorescent color of the fruit pulp (Wybraniec et al., 2002).

Pitaya fruit production has increased in recent years as a result of its nutritional and marketing value. It's being used as a source of functional materials to provide phytochemicals with strong antioxidant properties (Hossain *et al.*, 2021). Pitaya's robust phytochemical makeup fuels its antioxidant activity, which promotes general health and wellbeing by shielding cells from oxidative stress and lowering the chance of developing chronic illnesses. The fruit's wide range of antioxidants, which include carotenoids, vitamin C, polyphenols, and betalains, combine to combat free radicals and support cellular health. A balanced diet that includes pitaya can help boost immune system performance, lower the risk of illnesses linked to oxidative stress, and strengthen antioxidant defences. Pitaya's potential health benefits and uses in therapeutic and preventative nutrition will become clearer with more study into its bioactive ingredients and mechanisms of action (Singh & Kumar, 2023).

Hylocereus undatus fruit includes higher levels of minerals such as potassium, phosphorus, salt, and magnesium than other tropical fruits like the mango, mangosteen, and pineapple. It also contains higher amounts of vitamins, with vitamin C being the most abundant (Choo & Yong, 2011). Pitaya has an array of cancer fighting abilities due to its unique composition of bioactive compounds. Its anti-inflammatory, antioxidant, apoptosis-inducing, antiproliferative, angiogenesis-inhibiting, and DNA-protecting qualities are primarily responsible for its potential as an alternative anticancer medication. While these findings are promising, additional research including clinical trials is required to fully understand pitaya's mechanisms and efficacy in the prevention and treatment of cancer. (Wu *et al.*, 2006). In the present study, the antioxidant and anticancer property of *Hylocereus undatus* fruit pulp was studied and demonstrated its ability to kill skin melanoma causing cells in human body. The pitaya fruit (*Hylocereus undatus*) provides a wide range of health advantages as a result of its high nutritional content and diverse bioactive profile.

2. MATERIALS AND METHODS

Collection of Samples

The sample *Hylocereus undatus* fruit was collected from the local fruit market of my home town, Marthandam, Kanyakumari District, Soon after collecting the fruit, it was washed and cleaned. The cleaned white dragon fruit was cut and the peel was removed and then the pulp of the fruit was sliced in to small pieces and allowed to shade dry for a particular period of time and then it was ground and made into powder form. The sample was packed in a glass container and stored in room temperature for further analysis.

Preparation of crude extract

30 g of powdered plant Material was sequentially extracted using ethanol solvent in Soxhlet apparatus. After about forty siphons of each solvent extraction step, the materials were concentrated by evaporation.

2. Antioxidant Assay

DPPH radical scavenging Assay (Kevin *et al.*, 2013; Brand *et al.*, 1995)

Radical scavenging activity of the test sample against stable 2, 2-diphenyl 2-picrylhydrazyl hydrate (DPPH) was determined according to the method of Brand-William *et al.*, (1995) with slight modification. For DPPH assay the ascorbic acid was used as reference standard. The ascorbic acid stock solution was prepared in distilled water (1 mg/ml; w/v). A 60µM solution of DPPH in methanol was freshly prepared and a 200µl of this solution was mixed with 50µl of test sample at various concentrations (1.56, 3.12, 6.25, 12.5, 25, 50, 100, 200, 400, 800 µg/ml). The plates were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured at 515 nm. Control was prepared with DPPH solution only, without any extract or ascorbic acid. 95% methanol was used as blank.

Radical scavenging activity was calculated by the following formula;

$$\text{Percentage inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

In vitro Anticancer property (MTT Assay) (Joseph *et al.*, 2012)

In vitro anticancer activity Cell lines and culture conditions L929 cell lines (murine fibroblast) and SKMEL cell lines (human skin melanoma) were procured from NCCS, Pune, India. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, UK), 29 µg/ml L-glutamine, and 40 µg/ml Gentamicin. Cells were incubated in a humidified atmosphere of 5% CO₂ at 37°C.

Anti proliferative activity

The SKMEL cell lines (human skin melanoma) was maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum under standard conditions. The cytotoxic effect of the extract was determined using the MTT assay. Cells were treated with varying concentrations (6.25–100 µg/ml) of the ethanol fruit pulp extract of *H. undatus*, and IC₅₀ values were calculated. Decreased MTT was measured at 550 nm utilizing a microplate peruser (Das,

Italy). Control bunches got a similar measure of DMSO (0.1%). Untreated cells were utilized as a negative control while, cells treated with vincristine sulfate were utilized as a positive control

3. RESULT AND DISCUSSION

DPPH Radical Scavenging Assay

The antioxidant activity of *H. undatus* is mostly attributable to the presence of ascorbic acid (Vitamin C). Pitaya fruit contains vitamin C about 20.5 mg per 100 g (Elmarzugi, 2016). Ascorbic acid is a vitamin that has several physiological functions in living organisms, including acting as a reducing agent to protect cellular molecules from oxidative stress. Ascorbic acid can act as a scavenger for free radicals and oxygen-derived species such as singlet oxygen, hydroxyl radicals, and hydrogen peroxide during their oxidation. The regular consumption of pitaya fruit in human diets can help in the prevention and treatment of cough, sneezing, and asthma, as well as the improvement of wound healing as well as the promotion of liver functions (Elmarzugi, 2016).

In the present research, the antioxidant activity of ethanol fruit pulp extract of *Hylocereus undatus* has been studied by its ability to reduce DPPH. The result of reduction DPPH radicals produce the discoloration from purple colour to yellow pale colour which indicates the scavenging activity. The antioxidant activity ethanol fruit pulp extract of *Hylocereus undatus* was compared with the standard ascorbic acid at the concentration of (6.25, 12.5, 25, 50 and 100 mcg/ml). The ethanol fruit pulp extract of *Hylocereus undatus* showed 35.33 percentage of inhibition at the 100 mcg/ml concentration and the standard ascorbic acid showed 96.61 percentage of inhibition at the 100 mcg/ml concentration. The ethanol fruit pulp extract of *Hylocereus undatus* has very moderate antioxidant activity when compared to standard ascorbic acid. The results of DPPH activity of standard and ethanol fruit pulp extract of *Hylocereus undatus* were displayed in Table.1;2, and figure 1:2.

Table:1 DPPH Radical Scavenging Activity of Standard Ascorbic acid

| Concentration | Absorbance 1 | Absorbance 2 | Percentage of inhibition 1 | Percentage of inhibition 2 | Mean Percentage of inhibition |
|---------------|--------------|--------------|----------------------------|----------------------------|-------------------------------|
| 6.25 | 0.738 | 0.735 | 24.30 | 24.61 | 24.46 |
| 12.5 | 0.345 | 0.341 | 64.61 | 65.02 | 64.82 |
| 25 | 0.183 | 0.187 | 81.23 | 80.82 | 81.02 |
| 50 | 0.085 | 0.088 | 91.28 | 90.97 | 91.12 |
| 100 | 0.031 | 0.035 | 96.82 | 96.41 | 96.61 |
| IC 50 | 1.05 | | | | |

Table: 2 DPPH Radical Scavenging activity of ethanol fruit pulp extract of *Hylocereus undatus*

| Concentration | Absorbance 1 | Absorbance 2 | Percentage of inhibition 1 | Percentage of inhibition 2 | Mean Percentage of inhibition |
|---------------|--------------|--------------|----------------------------|----------------------------|-------------------------------|
| 6.25 | 0.934 | 0.931 | 4.20 | 4.51 | 4.35 |
| 12.5 | 0.855 | 0.857 | 12.30 | 12.10 | 12.20 |
| 25 | 0.744 | 0.741 | 24.00 | 24.00 | 23.84 |
| 50 | 0.680 | 0.684 | 29.84 | 29.84 | 30.00 |
| 100 | 0.633 | 0.628 | 35.58 | 35.58 | 35.33 |
| IC 50 | 136.71 | | | | |

Figure:1 Calibration Curve of DPPH Radical Scavenging Activity of standard Ascorbic acid and Ethanol fruit pulp extract of *Hylocereus undatus*

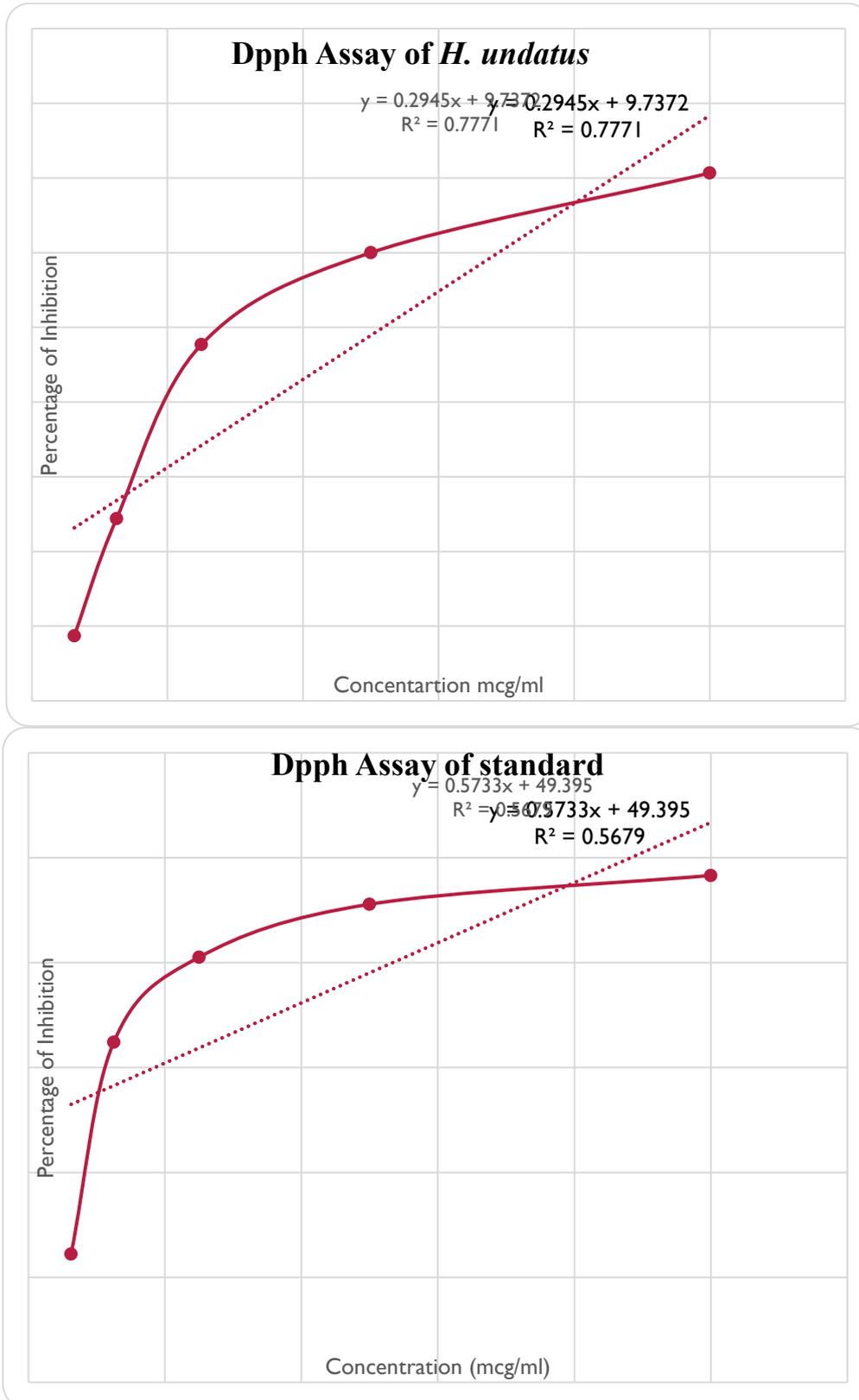


Figure 2. DPPH Radical Scavenging Assay of Standard Ascorbic acid and Ethanol fruit pulp extract of *Hylocereus undatus*



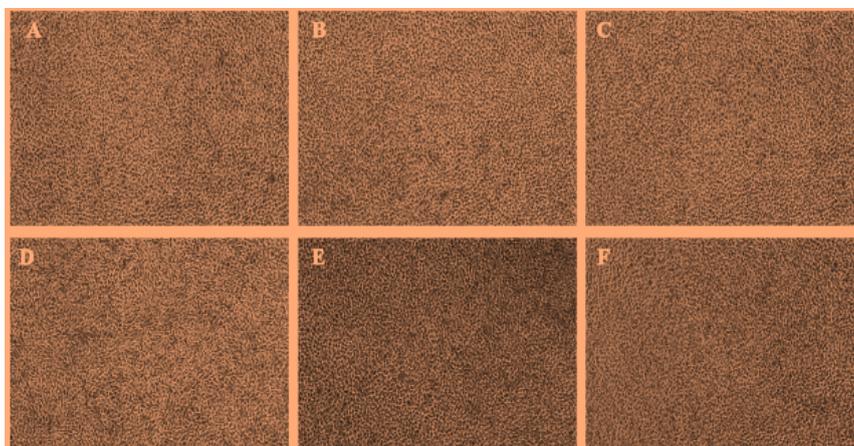
2. Anticancer Cytotoxic Effect of Ethanol fruit pulp extract of *Hylocereus undatus* on Cancer and Normal cell lines

Oxidative stress is one known component that leads to the growth of cancer. By absorbing radicals that are harmful and protecting cells from damage to their DNA, pitaya's antioxidants may mitigate this danger. *H. undatus* extracts have potent antioxidant properties due to their elevated levels of phenolic compounds. Pitaya inhibits oxidative damage, thereby helping halt the onset and spread of cancer. Inflammation that is persistent is a crucial element that also contributes significantly to the growth of cancer. Due to its anti-inflammatory effects, pitaya may help reduce the risk of cancer by modulating inflammatory pathways. *H. undatus* may have anticancer properties via preventing inflammatory reactions, as demonstrated by its ability to reduce inflammation markers in experimental mice (Padmavathy *et al.*, 2021).

Cytotoxic Activity of Ethanol fruit pulp extract of *Hylocereus undatus* on L929 cell line (Normal cell lines)

The L929 cell lines (murine fibroblast) were treated with ethanol fruit pulp extract of *Hylocereus undatus*. The percentage of cell viability of L929 cell lines treated with different concentrations of fresh fruit pulp extract of *H. undatus* (6.25 µg/ml, 12.5 µg/ml, 50 µg/ml, 100 µg/ml) and their cytotoxic activity was observed under an inverted phase contrast microscope. The cytotoxic activity of fresh fruit pulp extract of *H. undatus* showed no high dose dependent reduction in cell viability on L929 cells administered with different concentrations of fresh fruit pulp extract of *H. undatus*. The results indicated that the fresh fruit pulp extract of *H. undatus* was not cytotoxic to the normal cell lines. The results of cytotoxic activity of *A. muricata* were displayed in Figure 3 and Table 3.

Figure. 3. Morphology of L929 cells administered with different concentrations of the ethanol fruit pulp extract of *Hylocereus undatus*



(A) Control (B) 6.25 µg/ml (C) 12.5 µg/ml (D) 50 µg/ml (E) 100 µg/ml

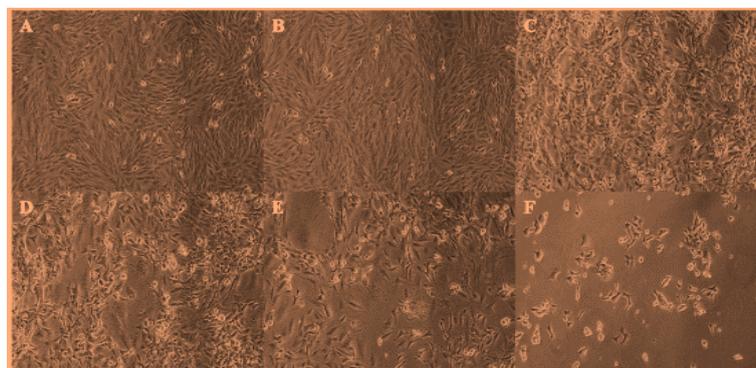
Table: 3 Average absorbance and Percentage of viability of ethanol fruit pulp extract of *Hylocereus undatus* L929 cell line (Normal cell)

| | | Ethanol fruit pulp extract of <i>Hylocereus undatus</i> | |
|--------|-----------------------|---|-------------------------|
| SI. No | Concentration (µg/ml) | Average absorbance | Percentage of viability |
| 1 | control | 0.712 | --- |
| 2 | 6.25 | 0.708 | 99.39±0.261 |
| 3 | 12.5 | 0.703 | 98.73±0.287 |
| 4 | 25 | 0.699 | 97.08±0.297 |
| 5 | 50 | 0.694 | 97.42±1.059 |
| 6 | 100 | 0.688 | 96.63±1.064 |

Cytotoxic Activity of ethanol fruit pulp extract of *Hylocereus undatus* SKMEL Cell line

Guimaraes *et al.*, (2017) looked at the anticancer effects of the pulp of *H. polyrhizus*. MCF-7 (ER+) cells examined with the pulp of pitaya showed a reduction in cell proliferation (500–1000 g mL⁻¹). The antiproliferative efficacy of pitaya fruit for the B16F10 melanoma cell was also demonstrated by Wu *et al.*, (2006). The antiproliferative potential of the pitaya peel extract was shown to be greater than that of the pulp extract on B16F10 melanoma cancer cells (Wu & Ng, 2008). In the present study, The SKMEL cell lines (human skin melanoma) were treated with ethanol fruit pulp extract of *Hylocereus undatus*. The percentage of cell viability of SKMEL cell lines treated with different concentrations of ethanol fruit pulp extract of *Hylocereus undatus* (6.25 µg/ml, 12.5 µg/ml, 50 µg/ml, and 100 µg/ml) and their cytotoxic activity against SKMEL cell lines was observed under an inverted phase contrast microscope. The cytotoxic activity of ethanol fruit pulp extract of *Hylocereus undatus* showed higher percentage of viability of 96 % at 6.25 µg/ml of fresh fruit pulp extract of *H. undatus* representing the average absorbance of 0.638, followed by 87.23±0.162, 73.76±0.394, 58.35±0.707, 38.87±0.865 percentage of viability in 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 100 µg/ml concentrations of ethanol fruit pulp extract of *Hylocereus undatus* showing the average absorbance range of 0.585, 0.485, 0.393 and 0.273. The results showed that higher the concentration of ethanol fruit pulp extract of *Hylocereus undatus* lower the viability of cell lines followed by higher the cytotoxic activity on cell lines. Dose dependant reduction in cell viability was observed in SK-MEL cells administered with different concentrations of the fresh fruit pulp extract of *H. undatus*. The IC₅₀ value of ethanol fruit pulp extract of *Hylocereus undatus* in SKMEL cell line was 72.32 µg/mL of the sample. The results were illustrated in and Table: 4. The graphical representation revealed the cytotoxic MTT assay activity of fresh fruit pulp extract of *H. undatus* on SKMEL cell lines and their results were represented in Figure: 4 and 5.

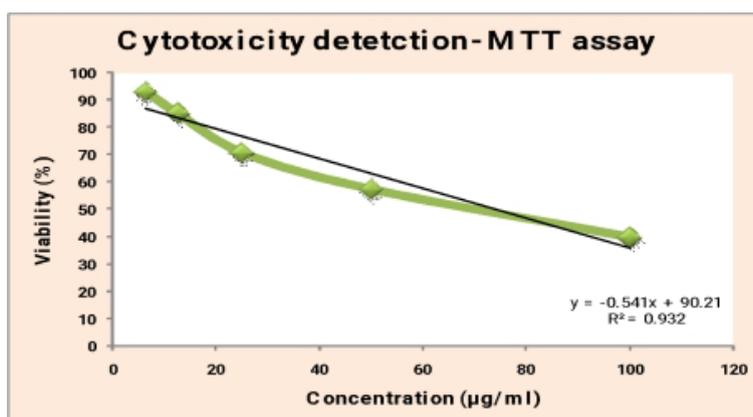
Figure.4 Morphology of SK-MEL cells treated with different concentrations of ethanol fruit pulp extract of *Hylocereus undatus* under inverted phase contrast microscope



(A) Control (B) 6.25 µg/ml (C) 12.5 µg/ml (D) 50 µg/ml (E) 100 µg/ml.

Table:4. Average absorbance values and Percentage of viability for varying concentration of ethanol fruit pulp extract of *Hylocereus undatus* SK-MEL cell.

| Sample | Concentration ($\mu\text{g/ml}$) | Average absorbance | Percentage of viability |
|---|------------------------------------|------------------------|-------------------------|
| Control | - | 0.686 | - |
| Ethanol fruit pulp extract of <i>H. undatus</i> | 6.25 | 0.638 | 96 \pm 0.006 |
| | 12.5 | 0.585 | 87.23 \pm 0.162 |
| | 25 | 0.485 | 73.76 \pm 0.394 |
| | 50 | 0.393 | 58.35 \pm 0.707 |
| | 100 | 0.273 | 38.87 \pm 0.865 |
| | IC ₅₀ | 72.32 $\mu\text{g/ml}$ | |

Figure.5. Graphical Representation of Cytotoxic activity of ethanol fruit pulp extract of *Hylocereus undatus* SK-MEL cells using MTT assay

Pasko *et al.*, 2021 on the cytotoxic activity of pitaya fruits against cancer and normal cells of skin, gastrointestinal and prostate accompanied by anti-inflammatory assay. They examined fruits from two different origins showed significant cytotoxic activity toward colon and prostate cancer cells, with no toxic effect on normal cells, with no anti-inflammatory effect. The *in vitro* anticancer activity of ethanol fruit pulp extract of *Hylocereus undatus* was studied by the cytotoxic impact of the different concentration of ethanol fruit pulp extract of *Hylocereus undatus* by MTT assay on L929 (murine fibroblast) cells and SK-MEL (human skin melanoma). Both the cell lines revealed varied cytotoxic activity representing the anticancer property of ethanol fruit pulp extract of *Hylocereus undatus*. The ethanol fruit pulp extract of *Hylocereus undatus* showed higher cytotoxic activity on SK-MEL (Skin melanoma cell line) representing lower viability compared to the L929 normal cell line depicting less cytotoxic activity and higher viability on normal cell lines.

4. CONCLUSION

Numerous high-nutrient food components can serve as anticancer molecules, assisting in the prevention of different types of cancers. The present study have reported that *Hylocereus undatus* fruit consumption could be valuable in the reduction of cancer risk. *Hylocereus undatus* fruit has an array of cancer fighting abilities due to its unique composition of bioactive compounds. Its anti-inflammatory, antioxidant, apoptosis-inducing, antiproliferative, angiogenesis-inhibiting, and DNA-protecting potentials are principally responsible for its potential as an alternative anticancer medication. The pitaya fruit, which has emerged as having tremendous potential due to its sugary taste, can be used in juices, ice cream, jams, and sweets, or it can be consumed in its natural state. The fruit's nutrient qualities and pulp color make it an appealing raw food for a variety of beverages, including fermented beverages and enzyme-based beverages.

CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest in this manuscript.

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