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Phytochemical Analysis, In-Vitro Antioxidant Activity and Normal Human Fibroblast Viability Study of Alcoholic Extract of The Dragon Fruit.

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Abstract

Red dragon fruit is a favored fruit, commonly utilized for both consumption and as a natural food dye. The peel and flesh of the red dragon fruit are rich in various antioxidant compounds, making them valuable for pharmaceutical and nutraceutical applications. This study assessed the phytochemical properties, antioxidant activities, and the impact of the alcoholic extract from the flesh of red dragon fruit on the viability of normal human fibroblasts. Antioxidant activity was measured using the DPPH assay, while the MTT assay was employed to evaluate the cell viability of normal human fibroblasts. Findings revealed that the alcoholic extract of H Polyrhizus fruit contained carbohydrates, alkaloids, phenolic compounds, tannins, saponins, glycosides, and flavonoids. The extract demonstrated significant antioxidant activity at 36.41%, and the extracts from the red dragon fruit flesh did not significantly affect fibroblast viability. The IC50 value for the alcoholic extract was 60.32±5.89 µg/mL, compared to IC50 values of 79.35±6.59, 55.01±4.59, 25.42±2.35, and 42.76±3.67 µg/mL for gallic acid, rutin, quercetin, and betanin, respectively. In summary, H Polyrhizus fruit exhibits strong antioxidant properties and could serve as a valuable source of natural compounds for new drug development, with its flesh extracts being relatively safe for normal cells compared to biomarkers.

Keyword: Antioxidant, cell viability, alcoholic extract, Phytochemical, Hylocereus Polyrhizus

INTRODUCTION

Hylocereus Polyrhizus, commonly known as Dragon fruit, is a notable tropical plant recognized for its substantial health benefits. The fruit's rising popularity is primarily due to its constituents, which include glucose, betanin, betalains, vitamins, organic acids, soluble dietary fiber, phyto albumins, and essential minerals [1]. Recently, the Government of Gujarat has renamed dragon fruit as KAMLAM (lotus) and has introduced incentives for its cultivation by farmers. This hardy plant is well-suited to various climates and soil types, particularly thriving in the semi-arid and arid regions of India. Following the initiatives of the Gujarat and Haryana Governments, the Central Government has decided to promote the cultivation of dragon fruit, often referred to as a “super fruit” due to its health benefits. The Central Government posits that, given the cost-effectiveness and global demand for the fruit, driven by its nutritional value, its cultivation can be expanded in India [2].

The dragon fruit, or Hylocereus spp., has recently garnered significant attention as a potential nutraceutical due to its numerous health benefits, which align with both traditional beliefs and contemporary scientific understanding [3]. Dragon fruits are rich in minerals such as potassium, magnesium, zinc, and phosphorus, and also contain smaller amounts of iron, calcium, and copper, all of which are beneficial due to their antioxidant properties. Extracts from the stems, petals, peels, and pulps of dragon fruit have demonstrated beneficial biological activity against diseases such as cancer, diabetes, obesity, and hyperlipidemia, as well as against pathogenic microbes including bacteria, fungi, and viruses [4,5]. Some researchers have specifically examined the benefits of red dragon fruit, including both its flesh and peel. The fruit flesh has been reported to be used in the treatment of diabetes, metabolic syndrome, and for the prevention of colitis and inflammation [6,7].

Herbal medicines are primarily utilized to facilitate healing by accelerating blood clotting and combating infections.[8] The precise side effects of herbal medicines remain undetermined due to the lack of standardization. A key consideration for the continued use of herbal medicines is the widespread availability of plant crude extracts, which offer diverse nutritional content and are economically more affordable compared to the purification of specific compounds.[9] The use of crude extracts, which contain multiple active substances, is believed to have a synergistic effect, resulting in a greater impact than the effect of each individual bioactive constituent, while also reducing toxicity.[10,11] Consequently, phytoconstituents from various plants in the form of crude extracts require further identification and standardization, with red dragon fruit peel (RDFP) being one such plant with potential for extraction.[12] The primary compounds in H. polyrhizus pulp include gallic acid, rutin, quercetin, and betanin. Gallic acid, a naturally occurring polyphenol, plays a significant role in various biological and therapeutic contexts. It is a potent antioxidant and free radical scavenger, protecting cells from oxidative stress. Beyond its antioxidant properties, gallic acid has shown promise in treating diseases, including cancer, by inducing apoptosis (programmed cell death) and interfering with cell signaling pathways. Additionally, it exhibits anti-inflammatory, antibacterial, and antidiabetic effects. Quercetin and rutin, which belong to the flavonoid group, reduce inflammation, oxidative damage, platelet aggregation, and inhibit cancer proliferation.[13] Betanin, a red pigment found in beets, serves multiple roles. It functions as a natural food colorant, a food preservative, and a potential antioxidant with health-promoting effects.[14] Conventional drugs, such as topical or systemic antibiotics, anti-inflammatory agents, immunomodulators, or other symptomatic treatments, are not entirely reliable due to the potential for undesirable side effects from long-term use and frequent exposure. Consequently, the treatment with "natural" ingredients or herbs is often considered an alternative, as bioactive components from plants are perceived as low risk and widely available. The various health benefits and limited utilization of RDFP have increased researchers' interest in converting the peel into products that are easier to use and have a longer shelf life, particularly for use as an alternative therapy.[15] The present study investigated the phytochemical analysis, in-vitro antioxidant activity, and normal human fibroblast viability test as a preliminary study to ensure the safe consumption of herbal medicines as a form of standardization.

Objective

The primary objective of this study is to evaluate the therapeutic potential of the alcoholic extract of *Hylocereus spp.* (dragon fruit) through Phytochemical Screening
To identify and qualitatively analyze the major classes of bioactive compounds such as phenolics, flavonoids, alkaloids, tannins, and betalains in the alcoholic extract of dragon fruit. In-Vitro Antioxidant ActivityTo assess the free radical scavenging potential of the extract using standard antioxidant assays including DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS, and FRAP methods. Normal Human Fibroblast Viability Study
To determine the cytotoxicity and biocompatibility of the extract on normal human fibroblast (NHF) cell lines using the MTT assay, ensuring its safety for potential therapeutic and cosmeceutical applications.

MATERIALS AND METHODS

Collection of plant

The study was conducted with Hylocereus polyrhizus at the Department of Pharmacy, Ganpat University, Mehsana, Gujarat. The Hylocereus polyrhizus fruit was procured from a local market in Gujarat, India, and its authenticity was verified by Dr. Nainesh R. Modi, Associate Professor in the Department of Botany, Bioinformatics & Climate Change Impacts Management, School of Science, Gujarat University, Ahmedabad, Gujarat, India.

Preparation of aqueous extract

500 gm of Hylocereus polyrhizus fruit pulp was weighed and subsequently defatted using n Hexane. The defatted material was then dried in the shade. Following this, it was extracted with 95% alcohol in a Soxhlet extractor. The alcoholic extract was allowed to dry.16-17.

Preliminary Phytochemical investigation

An investigation was conducted on an aqueous extract of Hylocereus polyrhizus. The analysis of common phytochemicals was performed using standard methodologies as outlined in "Practical Pharmacognosy" by Dr. C. K. Kokate and in the work of Trease and Evans. Various chemical assays were employed to identify the presence of chemical constituents, including carbohydrates, alkaloids, , phenolic compounds, tannins, saponins, glycosides, flavonoids.

Antioxidant activity

Total Phenol content (TPC)

The Folin-Ciocalteu method18 was employed to measure the total phenolic content in the plant extract. A 200 mL portion of the extracts (10 mg/mL) was mixed with 2.0 mL of solution A, which is composed of 10 mL of 2% Na2CO3, 0.1 mL of CuSO4, and 0.1 mL of sodium and potassium tartrate. After a 4-minute wait, 0.4 mL of 0.5 M sodium hydroxide was added. Ten minutes later, 0.2 mL of Folin-Ciocalteu reagent (diluted 1:1 v/v with water) was added. External calibration was conducted using different concentrations of gallic acid, namely 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mM. The solution was left to stand for 30 minutes before its absorbance was measured at 750 nm using a UV-Vis spectrophotometer. The total phenolic content was reported as mM gallic acid equivalent (mM GAE) using the gallic acid calibration curve.

In Vitro Antioxidant Activity

DPPH assay method

The antioxidant properties of the sample were assessed using the stable DPHH free radical method. A methanolic DPPH solution (0.05mM) (1000µL) was combined with 1000µL of an alcoholic extract at varying concentrations (20-100µL). The freshly prepared DPPH solution was stored in the dark at 4ºC. Subsequently, 96% methanol (2.7 mL) was added to the mixture, which was then shaken vigorously. The mixture was allowed to stand for 5 minutes, and its absorbance was measured spectrophotometrically at 517 nm. Methanol was used to set the absorbance to zero. A control sample with the same amounts of methanol and DPPH was also prepared. All tests were conducted in triplicate. The radical activity of the samples, expressed as a percentage of inhibition, was calculated using the formula

Percent (%) inhibition of DPPH activity = [(A-B)/A] ×100,

where A and B represent the absorbance values of the control and sample, respectively.

A graph of concentration versus percentage inhibition was plotted, and the concentration needed for 50% inhibition was determined19, 20.

Cell lines and culture

Human Embryonic Kidney (HEK) 293 cells were procured from the National Center for Cell Science (NCCS) in Pune and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS). The culture medium also included 5 ml of FBS, 0.5 ml of an antibiotic-antimycotic solution, and 1.25 ml of HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid].

MTT Assay for cell viability study[27-29]

Fibroblasts ($5x10^3$ cells/well) were cultured in a 96-well plate using DMEM supplemented with 10% FBS and subsequently incubated for 24 hours to establish a monolayer cell culture. The fibroblast cultures were then exposed to varying concentrations of red dragon fruit samples (10, 20, 40, 80, 160 µg/mL) and incubated for an additional 24 hours at 37°C. The viability of the fibroblasts was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at a wavelength of 570 nm. Control samples, which did not receive the extract solutions, exhibited 100% cell viability.

RESULT AND DISCUSSION

1. Phytochemical Investigation

The *Hylocereus polyrhizus* is rich with carbohydrates, alkaloids, , phenolic compounds, tannins, saponins, glycosides, flavonoids. The presence of phytochemicals is listed in **Table 1**.

2. Total Phenol content by Folin ciocalteu method

The total phenolic content was determined, with phenol identified as the most effective antioxidant. The Folin-Ciocalteu reagent was employed as the most effective analytical method for quantifying total phenol, using Gallic acid as the standard. From the calibration curve of Gallic acid (0.1 mg-0.8 mg/ml) (Figure 1), the calibration equation was established as $Y = 0.0601X + 0.0124$ ($R^2 = 0.9936$), where Y represents the absorbance value at 750 nm and X denotes the concentration of total phenolic content in mg/ml of the extract. An intense blue color was observed in the reaction mixture of fruit extracts following incubation. The data indicate that the flesh methanolic extract contains 18.33 GAE/100g (Table 2). Quantification of total phenol content demonstrates its antioxidant properties, with higher phenolic compound concentrations correlating with increased antioxidant activity.

3. Anti-oxidant activity by DPPH Assay

Antioxidant activity of extracts of red flesh dragon fruit by DPPH was carried out by determining IC_{50} of DPPH scavenging activities. IC50 of DPPH scavenging activities of extract were compared to IC_{50} of DPPH of standard ascorbic acid. Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight free radicals and protect us from various diseases. DPPH Assay for antioxidant activity display its antioxidant behavior. Inhibitory concentration 50% (IC_{50}) of DPPH scavenging activity of standard and extract solution was found to be 26.28% and 36.41%. Hence plant showed better antioxidant potential when compared to standard ascorbic acid by the DPPH method **(Figure 2)**. It means that the aqueous extract of a plant at a higher concentration captured more free radicals formed by the DPPH assay method.

MTT Assay for cell viability study

Human embryonic kidney (HEK) 293 cells were obtained from the National Center for Cell Science (NCCS) in Pune and grown in Dulbecco's Modified Eagle Medium (DMEM) with an addition of 10% fetal bovine serum (FBS). Fibroblasts were plated at a density of 5x10^3 cells per well in a 96-well plate containing DMEM with 10% FBS and allowed to incubate for 24 hours to form a monolayer culture. Following this, the fibroblast cultures were treated with different concentrations of red dragon fruit extracts (10, 20, 40, 80, 160 µg/mL) and incubated for another 24 hours at 37°C. The viability of the fibroblasts was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, with absorbance readings taken at 570 nm. The impact on cell viability is illustrated in Figures 3-7, and the percentage of cell viability, as determined by the MTT assay, is shown in Figure 8.

From MTT assay % cell viability effect of control with different concentration of biomarkers and Extract were compared. From comparision it was reveals that the extract has 60.32 µg/ml no any significant effect on cell viability. Cell viability study of all biomarkers and Extract were performed in different effects on the viability of Human Embryonic Kidney cell. The biomarkers and extract show cell viability in a concentration-dependent manner. Different preparation treatments of red dragon fruit flesh showed that the flesh caused no toxic effect on the Human Embryonic Kidney cell in the concentrations tested. Therefore, it can be concluded that the extract is relatively safer to normal cells.

Table 1 Phytochemical components present in extract of *Hylocereus polyrhizus*

Sr.no	Compounds	Alcoholic extract
1	Alkaloid	+
2	Flavonoids	+
3	Tannins and Phenol	+
4	Saponins	+
5	Steroids	+
6	Terpenoids	+
7	Glycosides	+
8	Carbohydrate	+

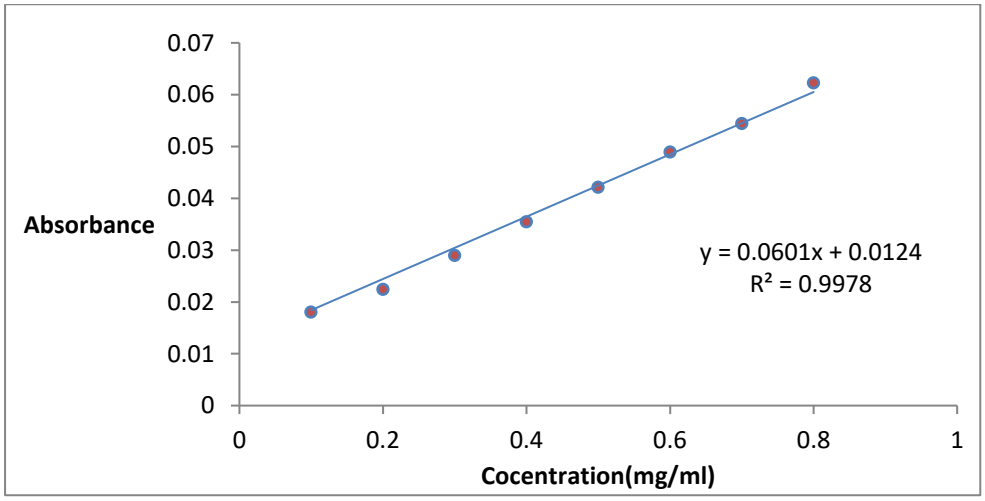


Figure 1: Total Phenolic Determination

Table 2: Data of Total Phenolic determination of Red Dragon Fruit extract

Sr no.	Absorbance	Concentration (mg/ml)	Total Phenol content (mg/gm)
1	0.0245	0.22	22
2	0.0238	0.19	19
3	0.0234	0.18	18
4	0.0227	0.14	14

5	0.0218	0.17	17
Average	0.02346	0.18	18
SD	0.00144	0.0260	2.60

Table 3: Antioxidant activity extracts and standard

Sr no.	**Concentration (µg/ml)**	**% Inhibition**	
		Extract	**Ascorbic acid**
1	20	46.09	43.40
2	40	58.53	51.44
3	60	72.34	61.72
4	80	78.08	69.58
5	100	81.99	78.70
6	IC_{50}	36.41	26.28

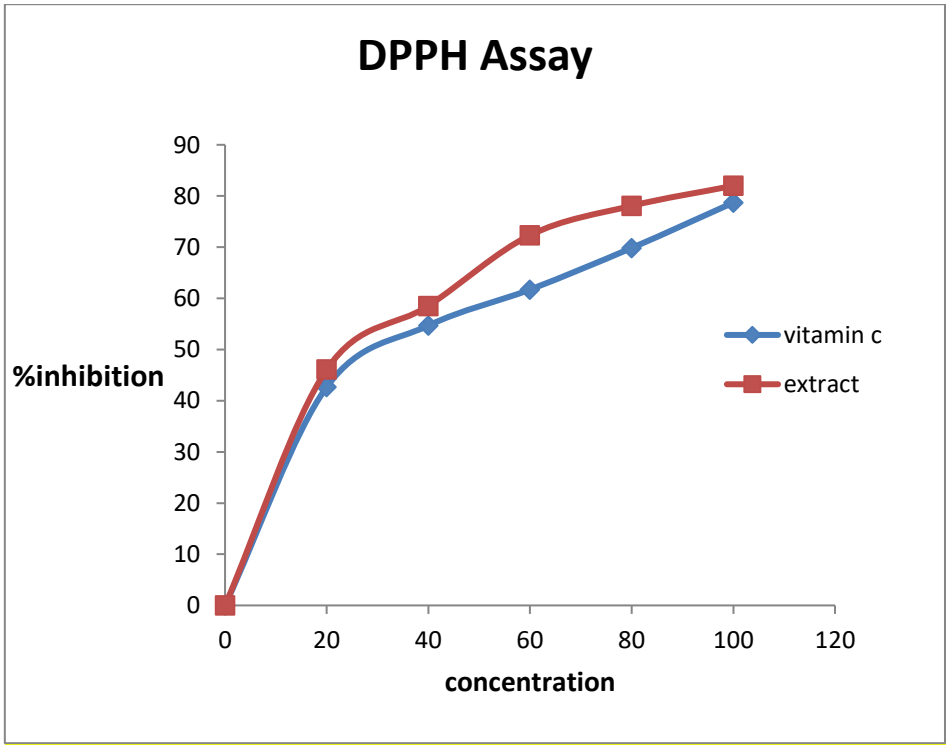


Figure 2: Antioxidant activities of extracts and standard

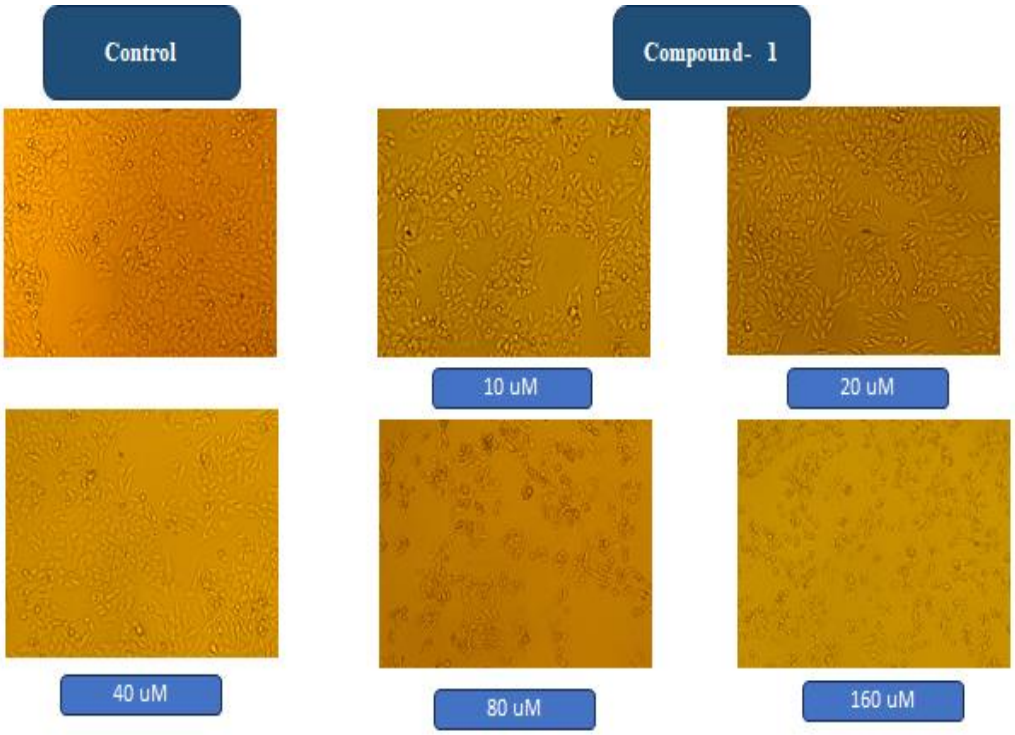


Figure 3: Cell viability effect of Gallic acid on HEK 293 cell lines

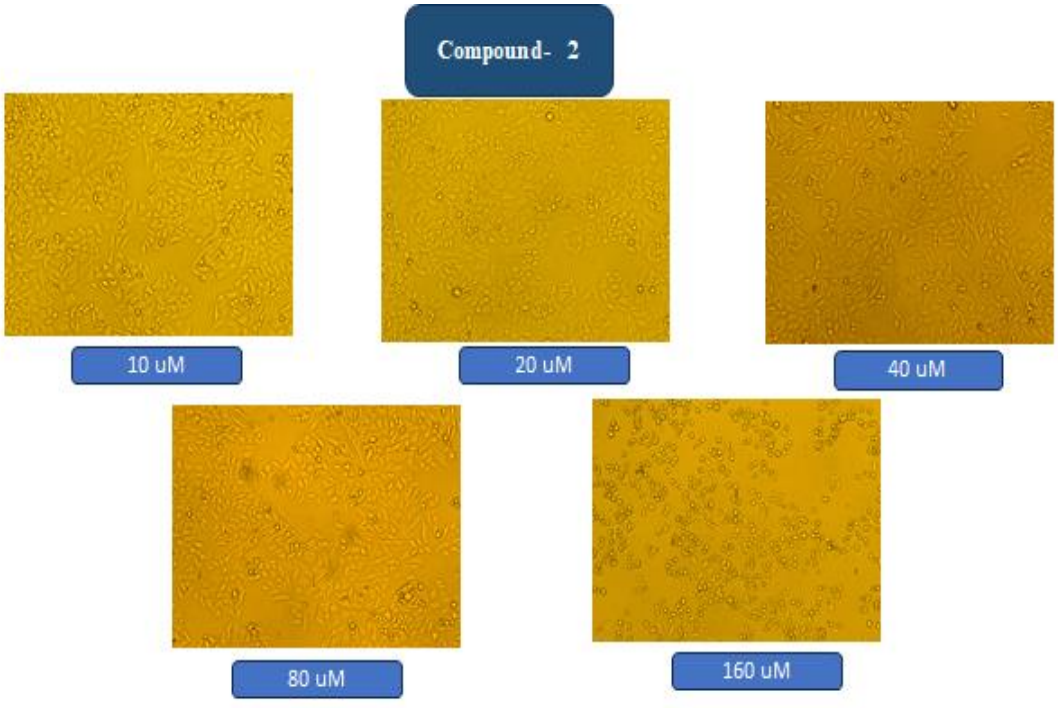


Figure 4: Cell viability effect of Rutin on HEK 293 cell lines

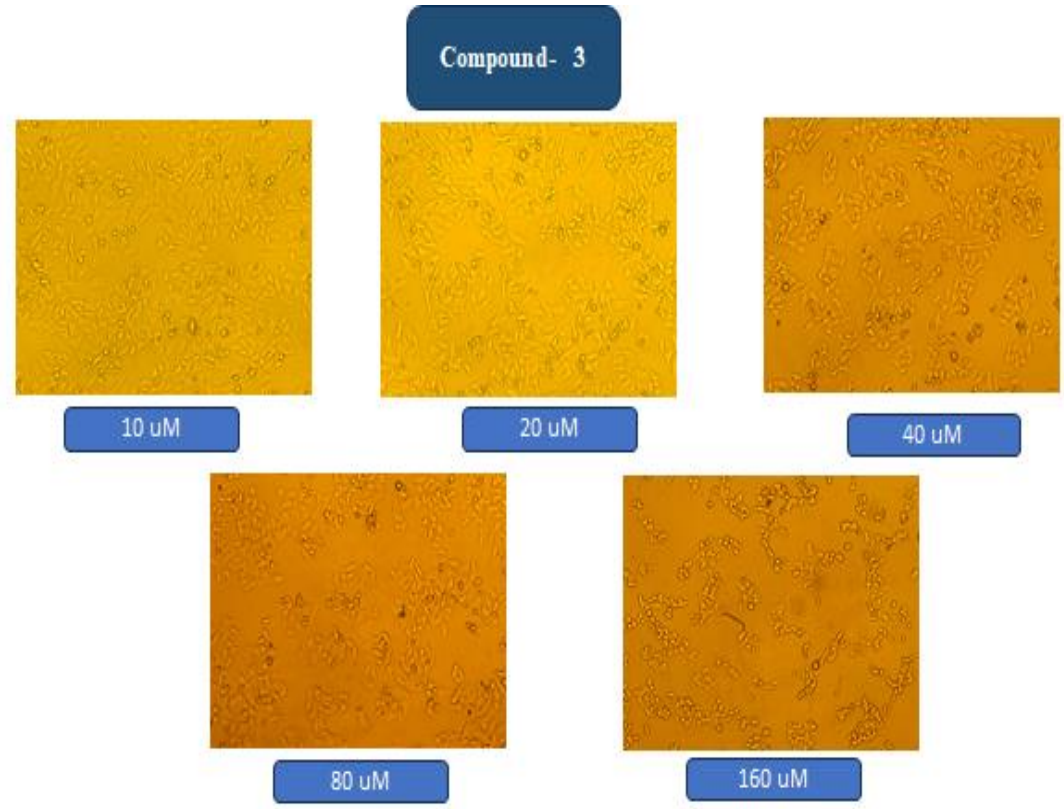


Figure 5: Cell viability effect of Quercetin on HEK 293 cell lines

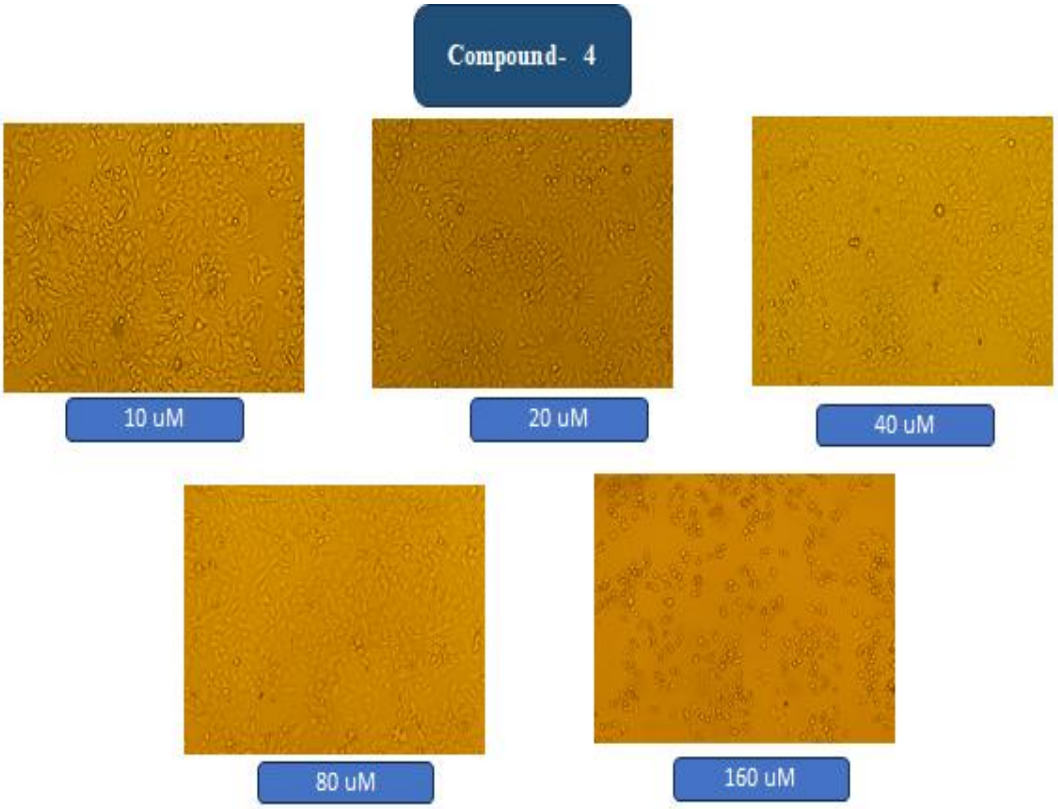


Figure 6: Cell viability effect of Betanin on HEK 293 cell lines

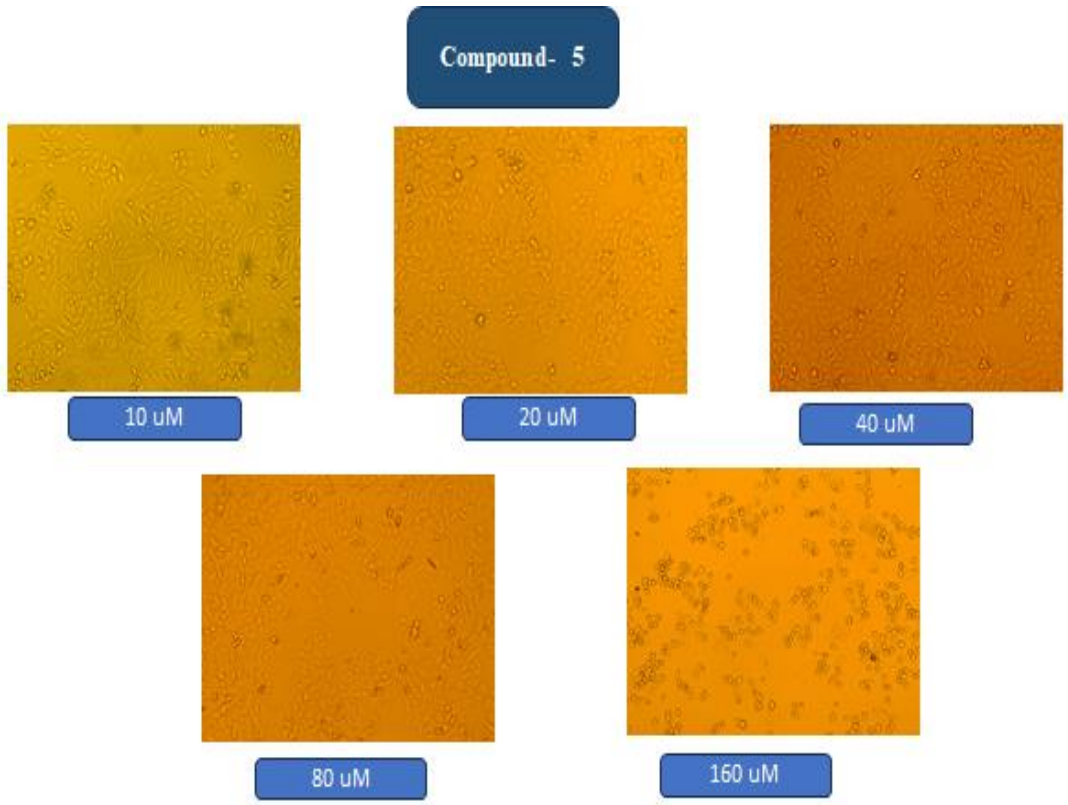
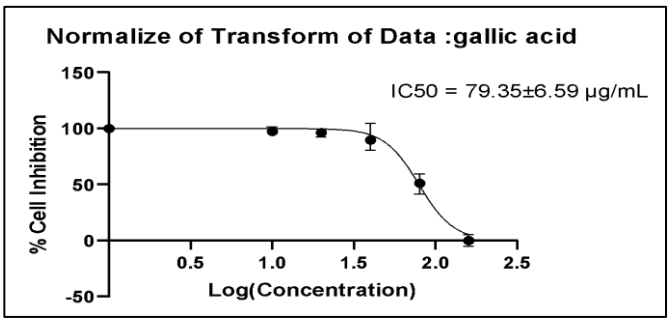
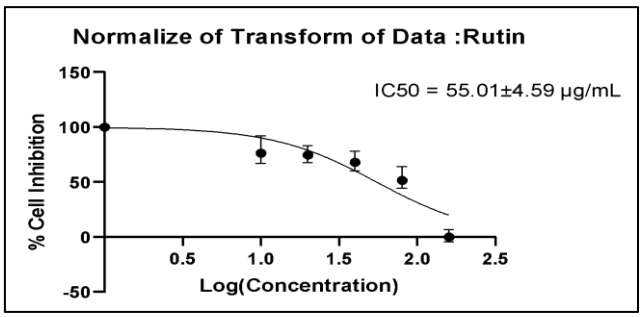
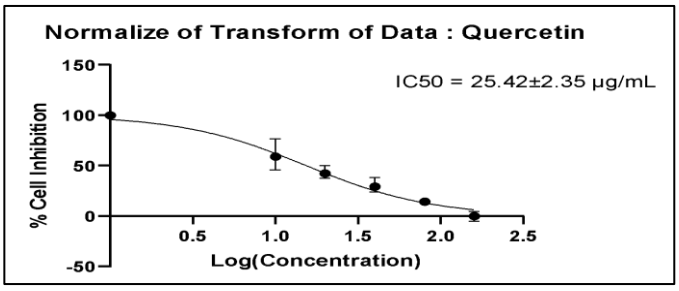
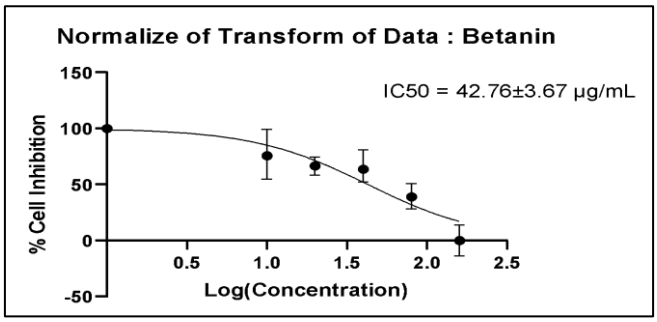


Figure 7: Cell viability effect of *H.polyrhizus* on HEK 293 cell lines









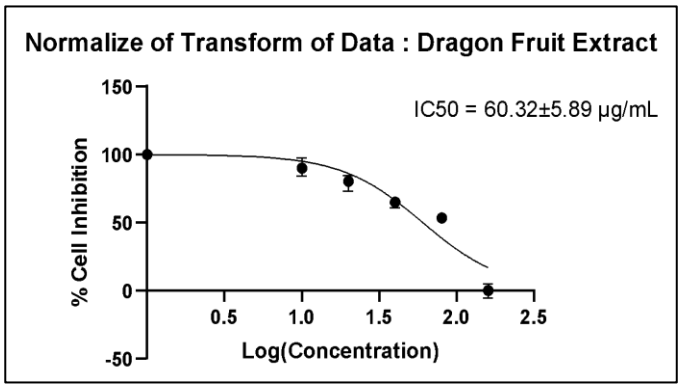


Figure 8: % Cell viability of Biomarkers and extract on HEK 293 cell line

Table 4: Comparision of Cell viability study of biomarkers and extract

Treatment	IC50
Gallic acid	79.35±6.59
Rutin	55.01±4.59
Quercetin	25.42±2.35
Betanin	42.76±3.67
Hydro alcoholic Extract	60.32±5.89

CONCLUSION

This study outlines a detailed approach for extracting and analyzing active compounds from dragon fruit, setting the stage for its use in the nutraceutical sector. The results emphasize dragon fruit's potential as a rich source of bioactive compounds, highlighting its significance in the nutraceutical industry. The measurement of total phenol content reveals its antioxidant properties, with a total phenol content of 18.33 GAE/100g. A higher concentration of phenolic compounds in an extract correlates with increased antioxidant activity. The DPPH assay for antioxidant activity demonstrates its antioxidant properties, with the extract solution's DPPH scavenging activity showing an inhibitory concentration 50% (IC50) of 36.41%. Various preparation methods of red dragon fruit flesh indicated that the flesh did not exhibit toxic effects on Human Embryonic Kidney cells at the tested concentrations. Consequently, it can be inferred that the nutraceutical enteric-coated tablet is relatively safe for normal cells. The study also highlights the significance of systematic extraction and thorough characterization in leveraging the therapeutic potential of natural resources. The successful identification and quantification of key compounds further validate the research, providing a solid foundation for future investigations. Thus, this research significantly contributes to the existing knowledge base, offering valuable insights for researchers and practitioners in the field.

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