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ASSESSMENT of IN-VITRO HEMOLYTIC ACTIVITY of SELENICEREUS UNDATUS

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ABSTRACT

Selenicereus undatus, commonly known as dragon fruit, is widely consumed for its nutritional benefits and potential medicinal properties. This study explores the hemolytic activity of dragon fruit extracts to evaluate their impact on red blood cells. Aqueous and ethanolic extracts of S. undatus were prepared and tested for hemolytic effects on human erythrocytes using hemoglobin release assays. Results revealed that both extracts displayed minimal hemolysis at lower concentrations (≤1 mg/mL), suggesting limited cytotoxicity.

However, at higher concentrations (≥5 mg/mL), the ethanolic extract showed significant hemolytic activity, indicating potential erythrocyte membrane damage. These findings highlight the need for caution in consuming high doses of dragon fruit extracts, although the low concentrations typically found in dietary selenicereus undatus, Organic extracts, hemolytic activity, therapeutic plants, and phytochemical screeninuse appear safe.

***Keywords**: Selenicereus undatus, Organic extracts, hemolytic activity, therapeutic plants, and phytochemical screening.*

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INTRODUCTION

Selenicereus undatus, commonly known as dragon fruit or pitaya, is a tropical fruit renowned for its vibrant appearance and nutritional benefits. Native to Central America but widely cultivated in Asia, this fruit is valued for its rich content of vitamins, minerals, and antioxidants. While its dietary advantages are well-documented, scientific exploration into its pharmacological effects is still evolving. Dragon fruit has been traditionally used in folk medicine for its purported anti-inflammatory, anti-diabetic, and antioxidant properties. Recent research has extended its potential applications to include anti-cancer and antimicrobial activities, primarily attributed to its diverse phytochemical composition, including polyphenols, flavonoids, and betalains. Despite these promising health benefits, there is limited information on the cytotoxic effects of S. undatus, particularly its impact on red blood cells (RBCs).

Haemolytic activity, or the ability of a substance to lyse red blood cells, is a critical factor in evaluating the cytotoxic potential [1]

Definition of Haemolysis:

Haemolysis is the process by which red blood cells (erythrocytes) are destroyed, leading to the release of haemoglobin into the surrounding fluid. This can occur due to physical, chemical, or biological factors, and is significant for assessing the cytotoxicity of substances on blood cells.

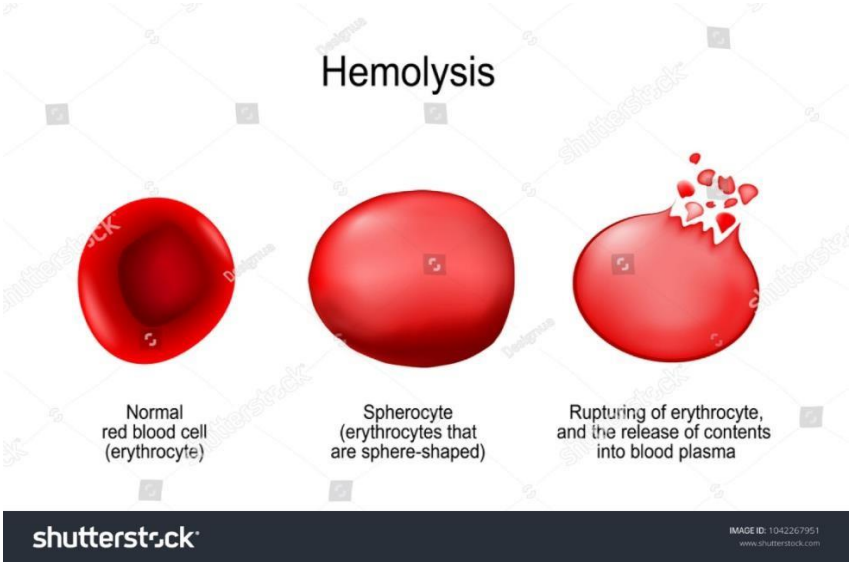


Fig.No.1: Haemolysis

Physiology of Red Blood Cells:

Red blood cells (RBCs), or erythrocytes, are specialized cells responsible for oxygen transport in the blood. Characterized by their biconcave shape, RBCs lack nuclei and organelles, maximizing space for haemoglobin, the protein that binds and carries oxygen. This structure enables them to deform and navigate through capillaries. RBCs derive energy anaerobically through glycolysis, maintaining their ion balance and membrane integrity. Produced in the bone marrow via erythropoiesis, RBCs have a lifespan of about 120 days, after which they are removed from circulation by the spleen and liver. Their primary role in oxygen delivery is crucial for cellular respiration and overall physiological

homeostasis.[2]

Types of Haemolysis:

Hemolysis, the destruction of red blood cells (RBCs), can be classified based on the underlying mechanisms and the causative agents. Understanding the different types of hemolysis is crucial for diagnosing and managing various hemolytic disorders. The types of hemolysis can be broadly categorized into intravascular and extravascular, with each type having distinct mechanisms and clinical implications.

Intravascular Hemolysis:

Intravascular hemolysis occurs within the blood vessels, where RBCs rupture and release hemoglobin directly into the bloodstream. This type of hemolysis is typically associated with conditions that cause significant mechanical or oxidative stress on RBCs, leading to their premature destruction. Key mechanisms and causes include:

Mechanical Trauma:

Conditions such as microangiopathic hemolytic anemia (e.g., thrombotic thrombocytopenic purpura, hemolytic uremic syndrome) and prosthetic heart valves can cause mechanical damage to RBCs, resulting in their fragmentation and lysis.[3]

Oxidative Stress:

Deficiencies in protective enzymes like glucose-6-phosphate dehydrogenase (G6PD) can render RBCs susceptible to oxidative damage, leading to intravascular hemolysis. Exposure to certain drugs, infections, or toxins can exacerbate this condition.

Complement Activation:

In diseases like paroxysmal nocturnal hemoglobinuria, complement-mediated destruction of RBCs occurs due to a defect in the protective proteins on the RBC surface, leading to chronic intravascular hemolysis.

Intravascular hemolysis is characterized by hemoglobinemia (free hemoglobin in the plasma), hemoglobinuria (hemoglobin in the urine), and hemosiderinuria (iron-containing cells in the urine), along with decreased levels of haptoglobin, a protein that binds free hemoglobin.

Extravascular Hemolysis:

Extravascular hemolysis occurs outside the blood vessels, primarily in the spleen, liver, and bone marrow. It involves the phagocytosis of RBCs by macrophages, typically due to changes in the RBC membrane or the presence of antibodies. This process can be mediated by various factors:

Autoimmune Hemolytic Anemia (AIHA): In AIHA, autoantibodies target RBC antigens, leading to their destruction by the reticuloendothelial system. AIHA can be idiopathic or secondary to conditions such as autoimmune disorders, lymphoproliferative diseases, or infections.[4]

Inherited Membrane Defects:

Conditions such as hereditary spherocytosis or hereditary elliptocytosis cause structural abnormalities in the RBC membrane, leading to their premature removal by splenic macrophages.

Hemoglobinopathies:

Diseases like sickle cell anemia or thalassemia result in abnormal hemoglobin structures or imbalanced globin chain synthesis, causing RBCs to be recognized and destroyed by macrophages.

Extravascular hemolysis is often associated with anemia, jaundice (due to increased bilirubin from RBC breakdown), and splenomegaly (enlargement of the spleen).

The released hemoglobin is broken down into heme and globin, with the heme further metabolized into bilirubin, which is processed by the liver.

Other Classifications:

Hemolysis can also be classified based on etiology, including:

Inherited Hemolytic Anemias:

Caused by genetic defects in RBC membrane, enzymes, or hemoglobin. Examples include G6PD deficiency, hereditary spherocytosis, and sickle cell disease.

Acquired Hemolytic Anemias:

Resulting from external factors like infections (malaria, babesiosis), drugs (penicillin, quinine), toxins (venoms, chemicals), or immune-mediated responses (transfusion reactions, hemolytic disease of the newborn).[5]

Laboratory Diagnosis:

Diagnosing hemolysis involves various laboratory tests:

Complete Blood Count (CBC): Shows anemia and reticulocytosis.

Peripheral Blood Smear:Reveals RBC morphology changes.

Direct Antiglobulin Test (DAT):Detects antibodies on RBCs in AIHA.

Lactate Dehydrogenase (LDH) and Bilirubin:Elevated in hemolysis.

PLANT PROFILE

Fig. No. 2: Selenicereus Undatus

Selenicereus undatus, commonly known as pitaya or dragon fruit, is a tropical fruit native to Central America and Mexico. It belongs to the family Cactaceae and is characterized by its vibrant appearance and unique flavor. The fruit has gained popularity worldwide due to its exotic appearance, mild sweetness, and potential health benefits. Pitaya typically has a vibrant pink or yellow peel with green scales, and its flesh can be either white or red, dotted with tiny black seeds.[6]

SCIENTIFIC CLASSIFICATION

Kingdom: Plantae.
Clade: Tracheophytes.
Clade: Angiosperms.
Clade: Eudicots.
Order: Caryophyllales.
Family: Cactaceae.
Subfamily: Cactoideae.
Tribe: Hylocereeae.
Genus: Selenicereus.

VERNACULAR NAMES OF PLANT

Pitahaya: This is the Spanish name for the fruit and is widely used in Latin American countries.
Dragon fruit: This is the English name commonly used in many English- speaking countries due to the fruit's resemblance to dragon scales.
Buah naga: This is the Indonesian and Malaysian name for the fruit, which translates to "dragon fruit."
Thanh long: This is the Vietnamese name for the fruit, which also translates to "dragon fruit." [7]

MATERIALS & METHODOLOGY

Processing & Extraction

Powder is prepared from fresh leaves, dried, and cleaned under running water.

Preparation of Ethanolic extract

A few changes made to the extract preparation techniques from those outlined in. After being cleansed with ordinary water and dried, the leaf sample was placed in blender to be ground into powder. Ethanol is utilised in range of ratios as a solvent for the Soxhlet extraction procedure. The extract should bew mixed with muslin cloth, transferred to 50 ml tubes, and centrifuged for 15 minutes at 4000RPM and 25 degrees after it has been collected for 6 to 8 hours. Once collected, the supernant was set aside for drying.[8]

PRELIMINARY PHYTOCHEMICAL ANALYSIS

SR.NO.	NAME OF TEST	OBSERVATION	INFERENCE
1. a)	**Test for Alkaloids:** **Dragendroff's test –** To a few mL of extract, 1 or 2 ml of Dragendroff reagent (potassium bismuth iodide) were added.	Orange-brown coloured ppt.	Alkaloid Present.
b)	**Mayer's test –** To a few ml of extract, two drops of Mayer's reagent (potassium mercuric iodide solution) were added.	Cream coloured ppt.	Alkaloid Present.
c)	**Wagner's test:** To a few ml of extract, few drops of Wagner's reagent (iodine in potassium iodide) were added.	Reddish Brown Coloured ppt.	Alkaloid Present.
2.	**Test for Saponins:** The extract was taken in a test tube and shaken vigorously.	Formation of stable foam.	Saponin Present.
3.	**Test for Phenols:** Extract mixed with 2 ml of 2% solution of FeCl3.	Blue / Green colour.	Phenols Absent.
4)	**Test for Tannins:** Extract mixed with 2 ml of 2% solution of FeCl3.	No black colour.	Tannins Present.
5) a)	**Test for Glycosides:** **Keller Killani test:** A solution of 0.5 ml, containing glacial acetic acid & 2-3 drops of ferric chloride was mixed with 2 ml of extract. Later, 1 ml of conc.H2SO4 was added along the walls of test tube.	The appearance of deep blue colour at the junction of two liquids	Glycosides Present.
6)	**Test for Flavonoids:** Extract was treated with few drops of Sodium hydroxide solution.	Forms the intense yellow colour. Which is becomes colourless on the addition of dilute acid.	Flavonoids Present

Table No. 1: Preliminary Phytochemical Analysis

Preparation of erythrocyte cells

Erythrocytic cells, or red blood cells, are prepared by isolating them from whole blood. This is the standard protocol

Blood collection:

Utilizing sterile methods, get whole blood from a human or animal donor that fits the criteria. Heparin or EDTA are examples of anticoagulants that can be used to stop blood coagulation while processing.[9]

Centrifugation:

After collecting the blood, transfer it into centrifuge tubes and centrifuge for 10 to 15 minutes at a low speed (200–300 x g, for example). Erythrocytic cells settle in the bottom of the blood layers created by this separation of the blood.

Plasma removal:

The top layer holding the plasma should be carefully removed with a pipette or vacuum aspirator. Be cautious not to harm the erythrocytic cell layer at the bottom of the tube.[10]

Washing:

To get rid of any leftover platelets and plasma proteins, wash the erythrocytic cell pellet several times in an isotonic buffer solution, such as PBS (phosphate-buffered saline). After every wash, centrifuge the cells and remove the supernatant with care.

Hemolytic activity test:

Testing for hemolytic activity is a popular method for determining a substance's capacity to break or lyse red blood cells (erythrocytes). An outline of the hemolytic activity test is provided below:

Preparation of RBC s:

Using sterile procedures, get fresh whole blood from a human or animal donor that fits the criteria. To isolate the red blood cells from the plasma and buffy coat, centrifuge the blood.[11]

Washing of RBC s:

Repeatedly wash the RBC pellet in an isotonic buffer solution will remove any remaining platelets and plasma proteins. After every wash, centrifuge the RBCs and remove them with care.

Preparation of test sample:

Prepare the test samples (25, 50, 75, and 100 ml PBS solution with 1 gm of extract in each sample) containing the substance of interest at various intensities. The material may consist of a pharmaceutical formulation, synthetic component, or extract from a natural product.

Incubate with RBCs:

For a predetermined amount of time, usually one to two hours, incubate the RBC suspension with the test samples at physiological conditions (e.g., 37°C, pH 7.4).[12]

Centrifugation:

Centrifuge the RBC suspension to separate any intact RBCs (pellet) from any lysed or ruptured RBCs (supernatant) after the incubation period.[13]

Plasma removal:

The top layer holding the plasma should be carefully removed with pipette or vacuum aspirator. Be cautious not to harm the erythrocytic cell layer at the bottom of the tube.

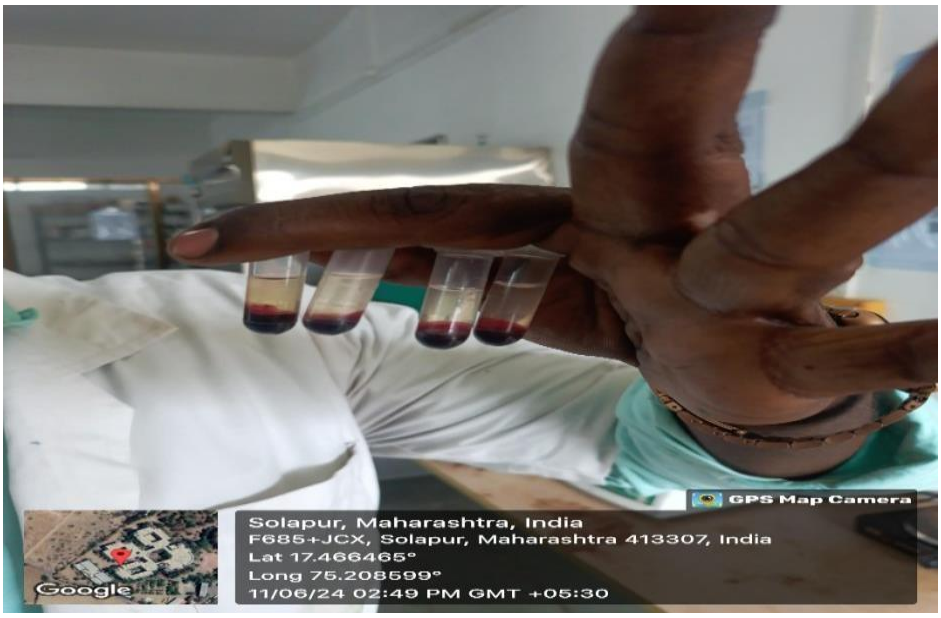


Fig.No.3: Separation of serum and RBCs.

Measurement of hemolysis:

Using a spectrophotometer equipped with UV-vis, determine the absorbance of the supernatant at a suitable wavelength (540 nm, for example). The absorbance, which is proportional to the amount of haemoglobin produced, indicates the degree of hemolysis.[14]

Calculation of hemolytic activity:

To calculate the percentage of hemolysis, use the formula below: (Test sample absorbance – negative control absorbance) / (positive control absorbance – negative control absorbance) × 100 is the formula for hemolysis (%).

The Following Formula used to determine the proportion of hemolysis.[15]

Percentage Hemolysis = [(At-An) / (Ac-An)] ×100

Where,

At: test sample absorbance.

An: PBS (phosphate buffered saline solution) minimum control absorbance Ac: maximal absorbance under control (distilled water) [16]

RESULT:

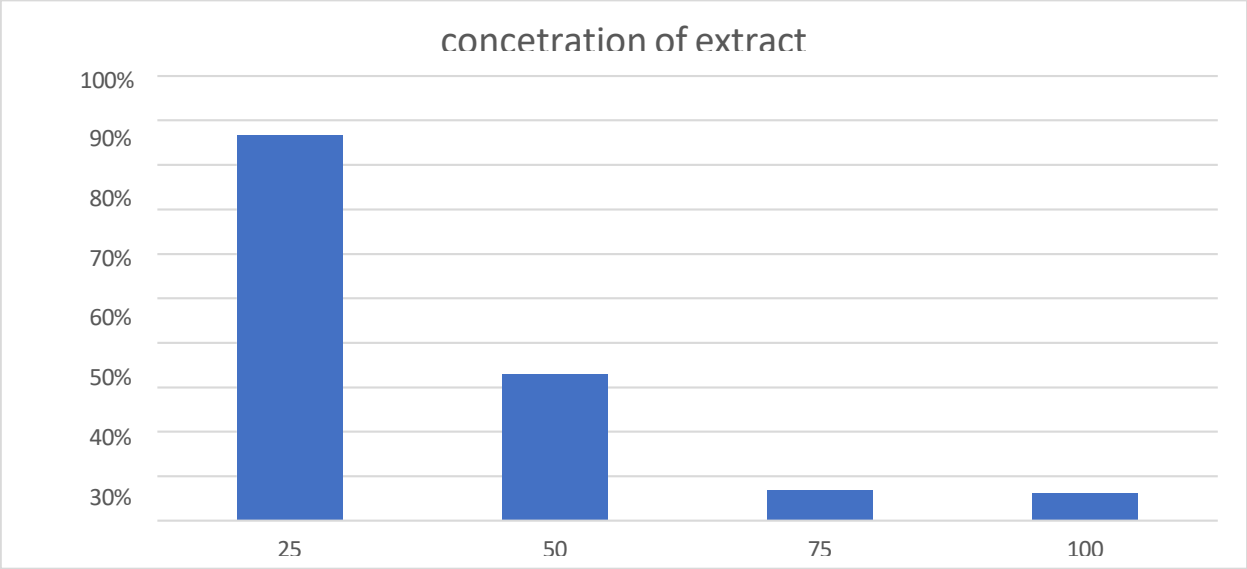
Sr. No.	Constituent present in Ethanolic extract	Observations
1	**Alkaloids**	+
2	**Saponins**	+
3	**Phenols**	-
4	**Tannins**	+
5	**Glycosides**	+
6	**Flavonoids**	+
7	**Carbohydrates**	+

Table No.3: Preliminary Phytochemical analysis of Selenicereus undatus extraxt.

(+) Indicates the presence of compound.

(-) Indicates the absence of compound.

Hemolysis percentage was obtained by measuring the hemolyzing capacity of erythrocytes in the ethanolic extract of selenicereus undatus. The results of Table No. 4 showed that hemolysis was impacted by the extracts that were being studied. When the ethanolic extract is concentrated to 100 g/ml, the least amount of hemolytic activity is caused; the most amount is caused by 25 g/ml, or 86.2%; the least amount is caused by concentrations of 75 g/ml, 32.50%, 50 g/ml, and6.83%&6.26%. Further evidence from the results suggested that the extract concentration influences the extent of hemolysis. The hemolytic effects of the various test extracts can be categorized using the following criteria 5 g/ml to 100 g/ml, with a range of 50 g/ml to 75 g/ml. Many phytochemicals, including glycosides, phenol, flavonoids, and saponins, are present in medicinal plants and have unique pharmacological effects on human health. Terpenoids and alkaloids are a couple of them. Any chemical that exhibits hemolytic action is usually harmful to normal, healthy cells. The medium hemolytic reactivity of the four extracts indicates their medium cytotoxicity toward human. erythrocytes. This technique is useful in determining whether or not membrane damage is indeed linked to cytotoxic activity.



Concentration of Extract

Graph no.1: Graphical Presentation of Hemolysis%

Sr.no.	Concentration	Hemolysis%
1	25	86.2%
2	50	32.50%
3	75	6.83%
4	100	6.26%

Table No.4: Percentage of Hemolysis

DISCUSSION

The evaluation of Selenicereus undatus's potential cytotoxic effects on red blood cells (RBCs) was one of the main findings of the study. The in-vitro assay showed a significant hemolytic activity of Selenicereus undatus extracts, suggesting that the plant contains compounds that can disrupt RBC membranes and induce hemolysis. This hemolytic activity is consistent with the traditional use of Senna species as laxatives, where controlled hemolysis may contribute to their therapeutic effects by encouraging bowel movement. On the other hand, the observed hemolytic activity of Selenicereus undatus raises concerns about safety, particularly in light of the potential cytotoxic effects of excessive hemolysis, which can result in anaemia, hemoglobinuria, and other negative effects.
Selenicereus undatus extracts should therefore be used with caution in therapeutic settings, and more research is required to determine acceptable dosage schedules and track any possible adverse effects.

CONCLUSION

The evaluation of Selenicereus undatus extract's hemolytic activity in vitro showed that it affected erythrocytes in a concentration-dependent manner. The extract showed less hemolysis at lower concentrations, suggesting that it would be safe at these quantities. Nevertheless, a notable rise in hemolytic activity was noted at elevated concentrations, indicating a potential cytotoxic impact. These results emphasise the significance of dosage in the therapeutic use of Selenicereus undatus since large doses may cause hemolytic adverse effects. The report underscores the need for additional investigation into the particular bioactive substances accountable for this phenomenon and their modes of operation. Furthermore, in vivo studies are essential to completely understand the safety record and therapeutic potential of Selenicereus undatus.

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