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ASSESSMENT OF IN VITRO HEMOLYTIC ACTIVITY OF ZORNIA GIBBOSA SPAN

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ABSTRACT

The assessment of in vitro hemolytic activity of Zornia gibbosa Span involves evaluating its potential to induce the lysis or rupture of red blood cells (erythrocytes) in a laboratory setting. Zornia gibbosa Span, commonly known as "marsh cudweed," is a plant species found in various regions, including tropical and subtropical areas. Research into its hemolytic activity can provide insights into its safety profile and potential pharmacological applications.

To assess hemolytic activity, the Zornia gibbosa Span extract is incubated with the red blood cell suspension under controlled conditions, such as temperature and pH. Following the incubation period, the mixture is centrifuged to separate intact red blood cells from any lysed or ruptured cells. The supernatant is then analyzed to measure the release of hemoglobin, a marker of hemolysis, using spectrophotometric methods.

The results are expressed as a percentage of hemolysis, calculated based on the absorbance of the supernatant compared to positive and negative controls. Higher absorbance values indicate greater hemolysis, while lower values suggest minimal or no hemolytic activity. Statistical analysis is often performed to assess the significance of the findings and determine dose-response relationships.

Keywords: *medicinal plants, organic extracts, hemolytic activity, zornia Gibbosa span, and phytochemical screening.*

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INTRODUCTION

Zornia gibbosa Span, a member of the Fabaceae family, is a plant species native to various regions, including Southeast Asia and South America. Traditionally, it has been utilized in folk medicine for its purported therapeutic properties. Recent studies have shed light on its potential pharmacological activities, including its hemolytic properties.Hemolysis, the rupture or destruction of red blood cells, is a crucial phenomenon in various physiological and pathological processes. Hemolytic agents can induce this process, resulting in the emission of intracellular components such as hemoglobin into the external surroundings. Understanding the hemolytic activity of natural compounds, such as those found in Z. gibbosa span, holds significance in both medical and biological research.Several studies have reported the hemolytic activity of Z. gibbosa span extracts, indicating its potential as a bioactive agent. However, the mechanisms underlying this activity and its implications remain to be fully elucidated. Investigating the hemolytic properties of Z. gibbosa span can provide insights into its pharmacological potential and facilitate the development of novel therapeutic agents.The goal of this review is to present a thorough summary of Z. gibbosa span's hemolytic activity.discussing its traditional uses, phytochemical composition, experimental findings, and potential applications in medicine. By synthesizing existing literature, this review seeks to stimulate further research into the pharmacological properties of Z. gibbosa span and its potential role in healthcare.[1] The assessment of in vitro hemolytic activity of Zornia gibbosa Span is a crucial aspect of understanding its safety and potential pharmacological effects. Zornia gibbosa Span, also known as "marsh cudweed," is a plant species found in various regions, including tropical and subtropical areas. Research into its hemolytic activity provides insights into its cytotoxic effects on red blood cells, which are essential for ensuring its safety profile in traditional medicine and pharmaceutical applications.The introduction of such a study typically outlines the importance of evaluating hemolytic activity to assess the potential toxicity of natural products. It highlights the significance of Zornia gibbosa Span as a medicinal plant and the need to investigate its biological properties comprehensively. Additionally, the introduction may discuss the relevance of hemolytic activity testing in drug discovery and development, emphasizing its role in early safety assessment.[2]

Definition of Hemolysis

The term "Hemolysis" describes the rupturing or lysis of erythrocytes, which allow to leave hemoglobin into the surrounding tissue.This phenomenon can occur due to various factors, including exposure to toxins, immune reactions, mechanical trauma, or biochemical alterations in the cell membrane. Hemolysis can cause intracellular contents to leak out of cells and the loss of functional Hemocyte, which can cause organ dysfunction and other clinical issues.[3]

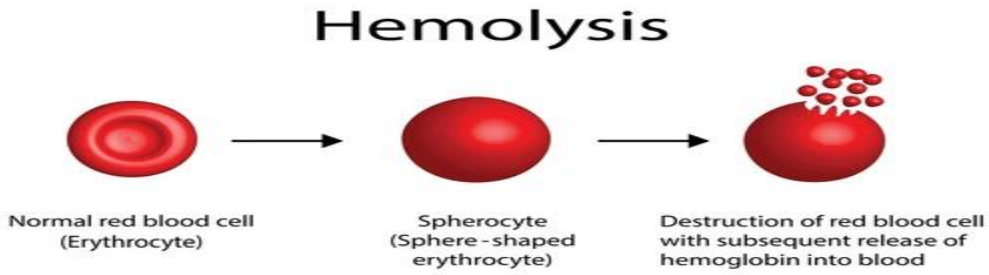


Figure No.1: Hemolysis

Why Does it occur

Mechanical Trauma

Physical damage to red blood cells, such as shear stress from turbulent blood flow, mechanical fragmentation in narrow blood vessels, or exposure to foreign objects, can lead to hemolysis. Conditions such as microangiopathic hemolytic anemia and mechanical heart valve prostheses are examples where mechanical trauma causes hemolysis.[4]

Chemical Agents

Exposure to certain chemicals or toxins can disrupt the integrity of the red blood cell membrane, leading to hemolysis. Examples include oxidative stress induced by reactive oxygen species, exposure to drugs or chemicals with hemolytic properties, or ingestion of certain toxins. For instance, drugs like quinine and certain antibiotics can cause hemolysis in susceptible individuals.[5]

Immune Reactions

Immune-mediated hemolysis occurs when antibodies or complement proteins target red blood cells, leading to their destruction. In cases of Rh or ABO incompatibility, hemolytic disease of the newborn (HDN), transfusion reactions, and other autoimmune disorders.

Infections

Some infectious agents, such as bacteria, viruses, or parasites, can directly invade red blood cells or produce toxins that cause hemolysis. malaria, which causes hemolysis of infected red blood cells, and certain bacterial infections that produce hemolytic toxins.

Genetic Disorders

Inherited genetic mutations affecting proteins involved in red blood cell structure or function can predispose individuals to hemolytic disorders.[6]

Types of Hemolysis

Intravascular Hemolysis: This is the breakdown of red blood cells inside blood vessels that takes place within the circulation. It may be brought on by illnesses like mechanical trauma, transfusion reactions, infections like malaria, or autoimmune hemolytic anemia.

Extravascular Hemolysis

In this kind, red blood cells are mostly destroyed in the liver and spleen, which are not part of the bloodstream. Extravascular hemolysis is frequently caused by illnesses like sickle cell disease, thalassemia, and hereditary spherocytosis.[7]

Osmotic hemolysis

When red blood cells are exposed to solutions with an abnormal osmotic pressure, they swell and burst. This condition is known as osmotic hemolysis. This is seen in diseases such as certain forms of anemia and hereditary spherocytosis.

Immune-mediated hemolysis

This happens when the immune system targets red blood cells for destruction because it perceives them as foreign.

Mechanical Hemolysis

When red blood cells are mechanically disrupted by physical trauma or shear forces, mechanical hemolysis takes place. This can occur during the implantation of an artificial heart valve, during extracorporeal circulation, or when blood flows through constricted arteries or surfaces.[8]

Mechanism of Hemolysis

Mechanical Hemolysis

Mechanical forces can physically disrupt RBCs, leading to their rupture and hemolysis.

Shear stress

High shear forces in turbulent blood flow, such as in stenotic blood vessels or artificial heart valves, can cause RBC fragmentation [9]

Trauma

Physical injury or trauma, such as crushing injuries or intravascular hemolysis due to mechanical heart valve prostheses, can directly damage RBCs.

Chemical Hemolysis

Exposure to certain chemicals or toxins can disrupt the integrity of the RBC membrane, leading to hemolysis.

Oxidative stress

Reactive oxygen species (ROS) generated during oxidative metabolism or exposure to environmental toxins can damage RBC membranes and induce hemolysis.[10]

Medications

Some drugs, such as certain antibiotics, antimalarials, and chemotherapeutic agents, have known hemolytic effects.

Immunological Hemolysis

Immune-mediated hemolysis occurs when antibodies or complement proteins target RBCs, leading to their destruction.

Autoimmune hemolytic anemia (AIHA)

Autoantibodies produced by the immune system target self-antigens on RBC membranes, leading to their destruction.[11]

Alloimmune hemolytic reactions

Transfusion reactions or hemolytic disease of the newborn (HDN) occur when antibodies produced by one individual (donor or mother) target RBC antigens in another individual (recipient or fetus).

Infectious Hemolysis

Some infectious agents, such as bacteria, viruses, or parasites, can directly invade RBCs or produce toxins that cause hemolysis.[12]

Hemolysis can have infectious cause such as:

Malaria

One of the life cycle stages of the malaria parasite is the infection and lysis of red blood cells. The release of merozoites from infected cells leads to the destruction of multiple RBCs, contributing to the characteristic symptoms of malaria, including anemia and hemolysis.[13]

Clostridium perfringens

This bacterium is known to produce toxins, including hemolysins, which can cause hemolysis. Infections with certain strains of Clostridium perfringens can result in hemolytic anemia, particularly in cases of gas gangrene and other severe infections.[14]

Babesiosis

Hemolysis and red blood cell destruction are the results of paramecieum called Babesia spp.'s infection. Tick bites carrying the babesiosis virus can infect humans and result in hemolytic anemia.[15]

Rickettsial Infections

Hemolysis can occur as a direct result of the pathogenic process in certain species of Rickettsia typhi, including Rickettsia rickettsii, which causes Rocky Mountain spotted fever. These infections can cause hemolytic anemia and other complications.[16]

Medication

Corticosteroids

Prednisone and prednisolone are frequently administered to treat autoimmune hemolytic anemia by suppressing the immune system and lowering the production of antibodies, which lowers hemolysis.

Immunosuppressive Medication

When corticosteroids alone are not enough to treat refractory cases of autoimmune hemolytic anemia, medications such as rituximab, cyclophosphamide, and azathioprine may be used. [17]

Folic Acid

To promote red blood cell production and offset increased turnover, folic acid supplementation is frequently advised in hemolytic anemias.

Immunoglobulins

To control the immune response and lessen hemolysis, intravenous immunoglobulin (IVIG) therapy may be utilized in severe cases of autoimmune hemolytic anemia.

Eculizumab

A monoclonal antibody called eculizumab inhibits the complement system, reducing hemolysis and preventing thrombosis in patients with paroxysmal nocturnal hemoglobinuria (PNH).[18]

Iron Supplements

To prevent or treat iron deficiency anemia brought on by increased red blood cell turnover, iron supplements may be required in chronic hemolytic anemias.

Blood Transfusions

In cases of acute or life-threatening hemolysis, transfusions of packed red blood cells may be necessary to treat severe anemia.

Chelating Agents

In hemolytic disorders linked to iron overload, such as transfusion-dependent thalassemia, chelating agents like deferoxamine or deferasirox may be administered. [19]

Hydroxyurea

In sickle cell disease, hydroxyurea can raise fetal hemoglobin levels and lessen the occurrence of hemolysis and vaso-occlusive crises.

Supportive Therapy

Hemolysis and related symptoms can be effectively managed with supportive measures like pain management, hydration, and avoiding triggers (such as certain medications, infections). [20]

MATERIAL AND METHODHOLOGY

Plant Collection and Authentication

The best time for collection of Zornia gibbosa span is generally during the flowering and fruiting seasons.Zornia gibbosa span plant were collected from Bhivghat ,sangli ,Maharashtra,India.The plant was authenticated by Mr. Suryavanshi M.Sc.B.Ed Botany plant physiology.

Plant Profile

Figure No.2: Zornia Gibbosa Span

Synonyms: Zornia saponacea.

Biological Source: Zornia gibbosa is a flowering plant that typically grows in tropical and subtropical regions. It is characterized by its small, clustered flowers and pinnate leaves.

Family: Fabaceae

Description

Color: The color of Zornia gibbosa Span varies depending on its growth stage and environmental conditions. In general, the plant exhibits green foliage with small yellow or white flowers when in bloom.

Odor: There is limited information available regarding the odor of Zornia gibbosa Span. Typically, plants in the Fabaceae family may have a mild or grassy scent.

Taste: Similarly, information about the taste of Zornia gibbosa Span is scarce. However, plants in the Fabaceae family often have a neutral or slightly bitter taste.[21]

Geographical Distribution

Zornia gibbosa Span is found in wetland habitats, including marshes, swamps, and along riverbanks, in regions with warm and humid climates.It is indigenous to several Central and South American nations, including Venezuela, Brazil, Colombia, Ecuador, Peru, and Peru.Additionally, Zornia gibbosa Span may be found in other tropical regions, such as parts of Africa and Asia.[22]

Cultivation

Zornia gibbosa Span is typically found growing in its natural habitat and may not be extensively cultivated for commercial purposes.Nonetheless, the plant can be grown in full sun or partial shade in moist, well-drained soil if cultivation is desired.

Propagation methods may include sowing seeds directly into the soil or transplanting seedlings into prepared beds.Adequate watering and soil fertility are important for the successful growth of Zornia gibbosa Span.

Collection

Collection of Zornia gibbosa Span may involve harvesting the aerial parts of the plant, including leaves, stems, and flowers, for medicinal or research purposes.

Harvesting should be done during the plant's active growing season when the desired phytochemical constituents are most abundant.

Care should be taken to avoid over-harvesting and to leave sufficient plant material for regeneration and ecological sustainability.

Proper drying and storage techniques should be employed to maintain the quality and efficacy of the collected plant material.[23]

Chemical Constituents

While specific chemical constituents may vary among different populations of Zornia gibbosa, phytochemical studies have identified various compounds in related species within the Zornia genus. These compounds may include alkaloids, flavonoids, saponins, and tannins. However, detailed chemical profiling of Zornia gibbosa specifically may require further research.[24]

Uses

Traditional uses of Zornia gibbosa are not extensively documented. However, species within the Zornia genus are known to have various ethnobotanical uses, including medicinal and cultural purposes. Some species are used in traditional medicine for treating conditions such as inflammation, fever, and gastrointestinal disorders. Additionally, Zornia gibbosa may have ecological significance within its native habitat, potentially serving as forage for grazing animals or contributing to soil stabilization[25]

Preparation of plant extracts Zornia Gibbosa span

Collection of Plant Material

Harvest fresh plant material of Zornia gibbosa Span from its natural habitat during the appropriate season when the phytochemical content is high.[26]

Cleaning and Drying

Thoroughly clean the harvested plant material to remove dirt, debris, and other contaminants.Air-dry the cleaned plant material in a well-ventilated area away from direct sunlight to prevent degradation of phytochemicals.

Grinding or Crushing

Once dried, grind the plant material into a coarse powder using a mortar and pestle, grinder, or blender. Ensure uniform particle size to facilitate extraction.[27]

Preparation of Ethanolic extract

Selection of Solvent

Depending on how polar the target compounds are, select an appropriate solvent or solvent mixture. Commonly used solvents include ethanol, methanol, chloroform, and water. A combination of solvents (e.g., ethanol-water) may be used for broad-spectrum extraction.

Extraction Procedure

Weigh a specific quantity of the dried and powdered plant material using an analytical balance. The quantity may vary based on the desired concentration and intended application of the extract.Place the weighed plant material in a clean and dry extraction vessel (e.g., Soxhlet extractor, Erlenmeyer flask).Add the selected solvent to the extraction vessel in an appropriate ratio (e.g., plant-to-solvent ratio of 1:10 to 1:20).Perform the extraction process using suitable methods such as maceration, Soxhlet extraction, or ultrasound-assisted extraction.Heat the extraction mixture gently if required, or agitate it periodically to enhance extraction efficiency.Continue the extraction process for a predetermined duration (e.g., 24 to 72 hours) to ensure maximum extraction of bioactive compounds.

Filtration and Concentration

After the extraction period, filter the extract using a suitable filter paper or membrane to remove solid residues and particulate matter.Concentrate the filtrate under reduced pressure using a rotary evaporator or by evaporation at low temperature to obtain a crude extract.Repeat the concentration process if necessary to achieve the desired concentration of the extract.

Storage

Store the prepared plant extract in amber-colored, airtight containers at appropriate temperatures (e.g., refrigerated conditions) to prevent degradation of phytochemicals.Properly label the containers with essential information such as the plant species, extraction solvent, concentration, and date of preparation.[28]

Preliminary phytochemical screening of Extraction

Preparation of phosphate buffer solution

Preparation of Phosphate Buffer Solution (0.1 M, pH 7.4):

Monobasic Potassium Phosphate (KH_2PO_4):

Weigh 13.6 grams of monobasic potassium phosphate (KH_2PO_4) using an analytical balance.

Dibasic Potassium Phosphate (K_2HPO_4):

Weigh 17.60 grams of dibasic potassium phosphate (K_2HPO_4) using an analytical balance.

Buffer Preparation

Fill a suitable container with about 800 mL of distilled water to dissolve the monobasic potassium phosphate.Slowly add the dibasic potassium phosphate to the monobasic potassium phosphate solution while stirring continuously.With the help of pH indicator strips or a calibrated pH meter, find out the solution's pH.Small amounts of monobasic or dibasic potassium phosphate solution can be added to the pH as needed to bring it up to the required 7.4.Make up the final volume to 1 liter with distilled water, and mix the solution thoroughly.

The process for making erythrocyte cells

Healthy willing donors of blood provided blood samples for collection. A PBS buffer, which is a sterile solution of saline phosphate was used to wash the pellet obtained after centrifuging 5 ml of blood. The cell suspension was added once more to a 0.5% solution of regular saline.

Perform the in-vitro hemolytic activity test

Make different concentration of solution and mixed with of the erythrocyte suspension and incubate and centrifuge it and free hemoglobin obtained, measured the absorbance using the UV-Vis spectrophotometer and calculate the % hemolysis.[29]

Phytochemical Investigation [30]

Sr No.	Name of Test	Observation	Inference
1.	**Test For Phenol:** Combine Extract with 2 milliliters of 2% $Fecl_3$	Blue/Green Colour solution.	Phenol present
2.	**Test for Saponin:** After placing the extract in a test tube, water was shaken briskly.	creation of a stable foam	Saponin present
3.	**Test for Tannins:** Combine Extract with 2% Fecl3	Black Colour	Tannin present
4.	**Test For Terpenoids:** Mixture of the extract and chloroform.then 2 ml of	Reddish brown colour observed in the interphase	Terpenoids absent

	concentrated sulfuric acid was added and gently shaken.		
5.	**Test for flavonoids:** Sodium hydroxide solution was added in small drops to treat the extract.	creation of a vivid yellow hue.which, upon adding diluted acid, turns colorless.	Flavonoids present
6.	**Test for Carbohydrates:** combine the extract with 2 ml of glacial acetic acid that has a few drops of 2% Fecl3. Transfer the mixture into a different tube that has 2 ml of concentrated sulfuric acid.	A brown ring at the interphase	Carbohydrate abesent
7.	**Test for protein:** The extract was exposed to a few drops of concentrated nitric acid.	Formation of yellow colour.	Protein present
8.	**Test for alkaloids:** **Dragndroffs test:** To a few ml of filtrate ,1 or 2ml of dragndroff reagent **Mayers test:** To few ml of extract ,2drops of mayers reagents . **Hagers test:** To few ml of extact 1or2ml of hagers reagent(saturated solution of picric acid)were added **Wagners test:** To few ml of the extract, few drops of wagner reagent (iodine in potassium iodide)	Orange brown coloured ppt. Cream coloured ppt . Yellow coloured ppt. Reddish brown coloured ppt.	Alkaloids present Alkaloids present Alkaloids present Alkaloids present

Table No.1: Phytochemical Screening of Zornia Gibbosa Span extract

EXPERIMENTAL WORK

Procedure

Preparation of Plant Extracts

Collection of Plant Material

Harvest fresh plant material of Zornia gibbosa Span, including leaves, stems, or other desired plant parts, from the natural habitat.

Preparation of Plant Material

Clean the harvested plant material to remove any dirt, debris, or extraneous material.

Dry the plant material thoroughly in the shade to remove moisture, preventing mold growth and degradation of phytochemicals.

Extraction Procedure

Weigh a specific quantity of dried plant material using an analytical balance. The quantity may vary depending on the experimental requirements and the desired concentration of the extract.Place the weighed plant material in a suitable extraction vessel (e.g., Soxhlet extractor, Erlenmeyer flask).Add an appropriate volume of solvent to the extraction vessel. Commonly used solvents include ethanol, methanol, or a mixture of water and organic solvent.Perform multiple extraction cycles using the chosen solvent to maximize the extraction efficiency and yield of bioactive compounds. The number of extraction cycles may vary, but typically 3-4 cycles are performed.Heat the extraction vessel using a

suitable method (e.g., Soxhlet extraction, maceration) to facilitate the extraction process. The temperature and duration of extraction depend on the solvent and the plant material.After each extraction cycle, collect the extract and filter it to remove any insoluble plant material or impurities.Combine the extracts obtained from multiple cycles and concentrate them using rotary evaporation or other suitable methods to obtain the desired concentration.[31]

Monobasic Potassium Phosphate (KH2PO4)

Weigh 13.60 grams of monobasic potassium phosphate (KH2PO4) using an analytical balance.Fill a suitable container (such as a glass beaker or flask) with about 800 mL of distilled water and dissolve the monobasic potassium phosphate.

Dibasic Potassium Phosphate (K2HPO4)

Weigh 17.40 grams of dibasic potassium phosphate (K2HPO4) using an analytical balance.Dissolve the dibasic potassium phosphate in approximately 200 mL of distilled water in a separate container.[32]

Buffer Preparation

Once both salts are completely dissolved, slowly add the dibasic potassium phosphate solution to the monobasic potassium phosphate solution while stirring continuously.

Use pH indicator strips or a calibrated pH meter to determine the solution's pH. When the pH reaches 7.4, add small amounts of monobasic or dibasic potassium phosphate solution to adjust the pH as needed. After adding distilled water to make the final volume to one liter, thoroughly mix the solution.[33]

Preparation of Erythrocyte Cell:

Preparation of erythrocytic cells, also known as red blood cells, involves isolation from whole blood. Here's a general procedure:

Blood Collection: Collect whole blood from a suitable animal species or human donor using sterile techniques. Use anticoagulants such as EDTA or heparin to prevent blood clotting during processing.

Centrifugation: Transfer the collected blood into centrifuge tubes and centrifuge at low speed (e.g., 200-300 x g) for 10-15 minutes. This separates the blood into layers, with erythrocytic cells settling at the bottom.

Plasma Removal: Carefully remove the upper layer containing plasma using a pipette or vacuum aspirator. Be careful not to disturb the erythrocytic cell layer at the bottom of the tube.[34]

Washing: Repeatedly wash the erythrocytic cell pellet in an isotonic buffer solution (phosphate-buffered saline, PBS) will ensure that any leftover platelets and plasma proteins are gone.Centrifuge the cells after each wash and carefully remove the supernatant.

Resuspension: After the final wash, resuspend the erythrocytic cells in the desired buffer solution or medium for further experimentation. Adjust the cell concentration as needed using a hemocytometer or automated cell counter.

Storage: Maintain the right temperature while storing the prepared erythrocytic cell suspension in aliquots. When storing erythrocytes for a short period of time, they are usually kept at 4°C, or frozen at -20°C or -80°C. Keep your cells intact by avoiding frequent freeze-thaw cycles.

Heamolytic Activity test:

Hemolytic activity testing is a common assay used to assess the ability of substances to cause the lysis or rupture of red blood cells (erythrocytes). Here's a general overview of the hemolytic activity test:

Preparation of Red Blood Cells (RBCs): Obtain fresh whole blood from a suitable animal species or human donor using sterile techniques. Centrifuge the blood to separate the RBCs from plasma and buffy coat.

Washing of RBCs: To get rid of any remaining plasma proteins and platelets, wash the RBC pellet several times in an isotonic buffer solution (such as phosphate-buffered saline, or PBS). Centrifuge the RBCs after each wash and carefully remove RBCs.

Preparation of Test Samples: Prepare the test samples containing the substance of interest at various concentrations.(25, 50, 50, 75, and 100 g/ml in the saline phosphate buffer) The substance could be a natural product extract, synthetic compound, or pharmaceutical formulation.

Incubation 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).

Centrifugation: After the incubation period, centrifuge the RBC suspension to separate the intact RBCs (pellet) from any lysed or ruptured RBCs (supernatant).

Measurement of Hemolysis: Measure the absorbance of the supernatant at a suitable wavelength (e.g., 540 nm) using a UV-vis spectrophotometer. The absorbance is directly proportional to the amount of hemoglobin released, indicating the degree of hemolysis.

Calculation of Hemolytic Activity: Use the following formula to determine the hemolysis percentage: Hemolysis (%) is equal to (test sample absorbance - negative control absorbance) / (positive control absorbance - negative control absorbance).[35]

RESULT

The Following Formula is used to Determine the proportion of hemolysis:

%Hemolysis = [(At-An)/(Ac-An)]×100

Where,

At: Absorbance of the test sample

An: Minimal control absorbance (Phosphate buffered saline PBS)

Ac: Maximum control absorbance (distilled water)

Observation

Sr. No.	Concentration	Absorbance	Hemolysis%	Protection
1.	25ml	0.008	96.01%	0.96
2.	50ml	0.084	58.28%	0.58
3.	75ml	0.106	4.72%	0.47
4.	100ml	0.315	3.06%	-0.56

Table No.2: Absorbance of sample

A helpful method for figuring out the molecular composition of various plant extracts and identifying bioactive compounds used in drug synthesis is phytochemical screening. Results of ethanolic extracts of Zornia Gibbosa Span leaves and stem subjected to phytochemical testing Zornia Gibbbosa Span screening revealed the presence of moisture as well as elemental substances such as nitrogen, sulfur, hydrogen, and carbon, but not reducing sugar.

These compounds indicate the plant's potential for medicinal purposes. Tests can be conducted to identify the various phenolic compounds, amino acids, and therapeutic value of the plant because neither the stem nor the leaf of the plant contain any reducing sugar.

Sr No.	Constituents	Observation
	Ethanolic Extract	
1	Saponins	+
2	Phenols	+
3	Tannins	+
4	Terpenoids	-
5	Flavonoids	+
6	Protiens	+
7	Carbohydrates	-
8	Alkaloids	+

Table No.3: Preliminary Phytochemical Screening of Zornia Gibbosa Span

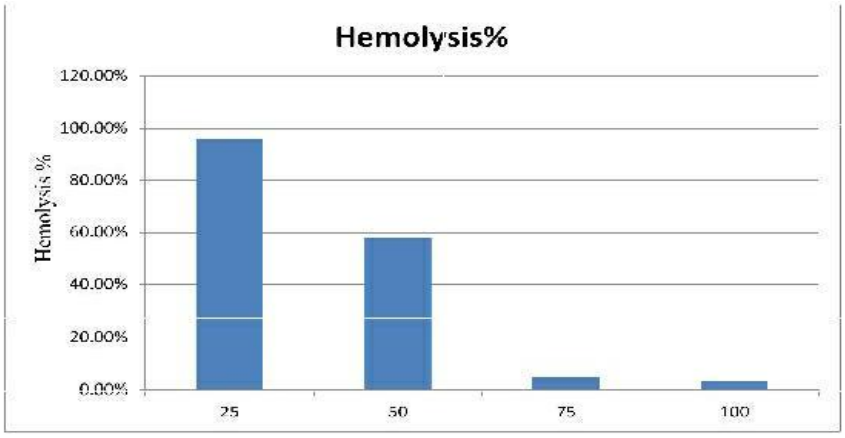
(-) indicates the absence of Compound

(+) indicates the presence of Compound

Erythrocytes were used to test the ethanolic extract of Zornia Gibbosa Span hemolyzing capacity: the results were expressed as a percentage of hemolysis. The findings (table no.4) demonstrated that hemolysis was impacted by the extracts under study. Concentrated at 100 g/ml, the ethanolic extract exhibits the least hemolytic activity: at 75 g/ml, 4.72%, 50 g/ml, and 58.28%, the least hemolytic activity is produced: at 25 g/ml, or 96.01%, the most hemolytic activity is produced. The outcomes also showed that the extract concentration affects the extent of hemolysis. The various test extracts' hemolytic effects can be categorized using the following criteria: 25 g/ml to 100 g/ml, with a range of 50 g/ml to 75 g/ml. Many phytochemicals, including terpenoids and alkaloids, are present in medicinal plants and have distinct pharmacological effects on the human body. Examples of these components are flavonoids, phenol, saponin, and glycosides. Any substance that exhibits hemolytic activity is likely cytotoxic to healthy, normal cells in general. The four extracts' medium hemolytic response reveals their medium cytotoxicity toward human erythrocytes. This assay is useful in determining whether or not membrane damage is actually linked to cytotoxic action.

Sr No.	Concentration	Hemolysis %
1.	25	96.01%
2.	50	58.28%
3.	75	4.72%
4.	100	3.06%

Table No.4: Percentage of Hemolysis



Graph No.1: Graphical Presentation of Hemolysis %

DISCUSSION

The evaluation of in-vitro hemolytic activity of Zornia gibbosa Span represents a significant step in understanding the potential physiological impact of this plant extract on red blood cells. Hemolysis, the breakdown of red blood cells, is a critical parameter in assessing the cytotoxicity and safety profile of natural products, including medicinal plants like Zornia gibbosa Span.

Conducting in-vitro studies involves exposing red blood cells to varying concentrations of the plant extract and measuring the extent of hemolysis. This process allows researchers to gauge the extract's potential to disrupt the integrity of erythrocytes, providing insights into its cytotoxic effects and potential therapeutic applications.

The discussion surrounding the research on Zornia gibbosa Span's hemolytic activity should consider several key aspects. Firstly, understanding the concentration-dependent nature of hemolysis is crucial for determining any dose-response relationships. This involves assessing how different concentrations of the plant extract affect the degree of hemolysis, providing insights into its potency and safety margins.

Furthermore, identifying the specific bioactive compounds responsible for the observed hemolytic effects is essential. Zornia gibbosa Span contains a diverse array of phytochemicals, and pinpointing the active constituents can help elucidate the underlying mechanisms of hemolysis. This knowledge is valuable for both pharmacological research and ensuring the safe use of Zornia gibbosa Span in traditional medicine practices.

Moreover, the implications of the findings on human health and therapeutic applications should be thoroughly discussed. While hemolysis can indicate cytotoxicity, it may also suggest potential pharmacological benefits, such as antimicrobial or anticancer properties. Therefore, a comprehensive understanding of Zornia gibbosa Span's hemolytic activity is essential for evaluating its therapeutic potential and safety profile.

In conclusion, the assessment of in-vitro hemolytic activity of Zornia gibbosa Span provides valuable insights into its biological effects and potential medicinal applications. Further research exploring its mechanisms of action, bioactive constituents, and in-vivo effects is necessary to fully harness its pharmacological benefits while ensuring its safety for human use.

CONCLUSION

The in-vitro hemolytic activity assessment of Zornia gibbosa involves evaluating its potential to cause hemolysis, or the destruction of red blood cells. This is an important test to determine the safety of plant extracts for therapeutic use. The procedure typically involves exposing red blood cells to various concentrations of the plant extract and then measuring the degree of hemolysis, which can be quantified by the release of hemoglobin into the surrounding solution.

Studies have shown that Zornia gibbosa possesses significant bioactive properties, including antioxidant and anti-hemolytic activities. The hemolytic assay results indicate that at certain concentrations, the extracts do not induce significant hemolysis, suggesting that they are safe for red blood cells at these levels. This aligns with findings that Zornia gibbosa extracts have robust antioxidant properties, which contribute to their protective effects against oxidative damage in erythrocytes.

In conclusion, Zornia gibbosa demonstrates low hemolytic activity in vitro, indicating potential safety for further pharmacological applications. However, comprehensive in vivo studies are necessary to confirm these findings and to fully establish the therapeutic safety profile of this plant extract.

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