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ASSESSMENT OF IN VITRO HEMOLYTIC ACTIVITY OF SENEGALIA PENNATA

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ABSTRACT

Senegalia pennata, a medicinal plant rich in bioactive compounds, has gained attention for its potential therapeutic properties. This study aimed to evaluate the in-vitro heamolytic activity of S. pennata extract. Fresh leaves of S. pennata were collected and extracted using standard procedures. The extract was then subjected to hemolytic activity assessment using erythrocytes obtained from healthy donors. Various concentrations of the extract were incubated with erythrocytes, and the degree of hemolysis was measured spectrophotometrically.Results showed a concentration-dependent hemolytic activity of S. pennata extract. At lower concentrations, minimal hemolysis was observed, while at higher concentrations, a significant increase in hemolytic activity was noted. This suggests a dose-dependent effect of the extract on erythrocytes. Furthermore, the hemolytic activity was found to be influenced by factors such as pH and temperature. this study demonstrates the hemolytic potential of Senegalia pennata extract in vitro. Further investigation into the underlying mechanisms and in vivo studies are warranted to elucidate its therapeutic relevance and safety profile.

Keywords: *senegalia pennata, Phytochemical screening, hemolytic activity, medicinal plant, organic extracts.*

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INTRODUCTION

Hemolysis refers to the rupture or destruction of red blood cells (erythrocytes), leading to the release of hemoglobin into the surrounding fluid. This phenomenon can be induced by various factors including toxins, drugs, and certain plant extracts. Understanding the hemolytic activity of natural substances, particularly medicinal plants, is crucial for assessing their safety and potential therapeutic applications. Senegalia pennata, formerly known as Acacia pennata, is a shrub belonging to the Fabaceae family, commonly found in tropical and subtropical regions of Asia. It has been traditionally used in folk medicine for the treatment of various ailments due to its rich phytochemical composition. Previous studies have reported its anti-inflammatory, antioxidant, antimicrobial, and anticancer properties. However, despite its potential therapeutic benefits, the hemolytic activity of S. pennata extract remains relatively unexplored. The assessment of hemolytic activity involves exposing erythrocytes to a substance of interest and measuring the extent of red blood cell lysis. This can be performed using in-vitro assays, which offer a controlled environment to study the effects of the test compound on erythrocyte integrity. In-vitro hemolysis assays provide valuable information regarding the cytotoxicity and potential harmful effects of substances on blood cells.[1]

Several factors influence the hemolytic activity of plant extracts, including their chemical composition, concentration, and physicochemical properties. The presence of bioactive compounds such as saponins, alkaloids, flavonoids, and tannins in plant extracts can contribute to their hemolytic potential. Additionally, environmental factors such as pH and temperature can modulate the interaction between the extract and erythrocytes, influencing the degree of hemolysis. Understanding the hemolytic activity of S. pennata extract is essential for assessing its safety profile and potential use in therapeutic applications. Hemolysis can lead to various adverse effects, including anemia, organ damage, and systemic toxicity. Therefore, thorough evaluation of the hemolytic properties of S. pennata extract is necessary to ensure its safe utilization in medicine. Previous studies have investigated the hemolytic activity of related species within the Acacia genus, such as Acacia arabica and Acacia catechu. These studies have reported varying degrees of hemolytic potential, with extracts exhibiting concentration-dependent effects on erythrocytes. However, the specific hemolytic activity of S. pennata extract remains to be elucidated. [2]

In this study, we aimed to assess the in-vitro hemolytic activity of Senegalia pennata extract using standardized protocols. Fresh leaves of S. pennata were collected and subjected to extraction to obtain the crude extract. The extract was then evaluated for its ability to induce hemolysis using erythrocytes obtained from healthy donors. The hemolytic activity assay involved incubating erythrocytes with varying concentrations of S. pennata extract and measuring the release of hemoglobin into the surrounding medium. Spectrophotometric analysis was used to quantify the degree of hemolysis, with higher absorbance values indicating increased erythrocyte lysis. Furthermore, the influence of environmental factors such as pH and temperature on the hemolytic activity of S. pennata extract was investigated. These parameters were varied to simulate physiological conditions and assess their impact on erythrocyte integrity. understanding the hemolytic activity of Senegalia pennata extract is essential for evaluating its safety and potential therapeutic applications. By elucidating the mechanisms underlying its interaction with erythrocytes, this study contributes to the broader understanding of the pharmacological properties of S. pennata and facilitates its rational use in medicine.[3]

Definition of Hemolysis

Hemolysis is the process of red blood cell destruction, leading to the release of hemoglobin into the surrounding fluid. It can be induced by various factors such as toxins, drugs, and certain plant extracts. Hemolysis can have adverse effects on health, including anemia and organ damage.

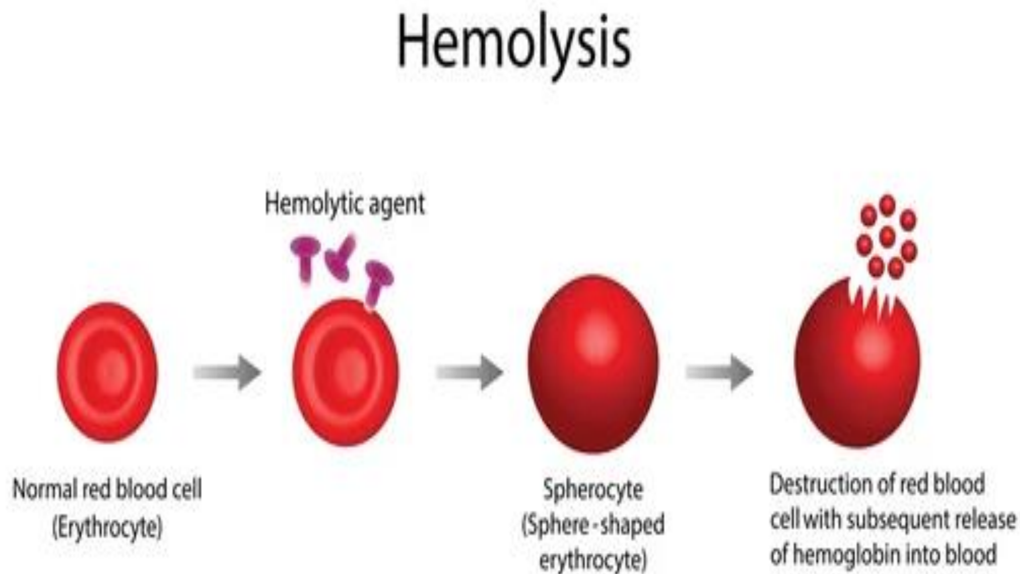


Figure No.1: Hemolysis

Why Does it occur

Toxins

Chemical substances such as drugs, heavy metals, and industrial chemicals can damage red blood cells leading to their rupture and hemolysis.

Infections

Microorganisms like bacteria, viruses, and parasites can directly attack red blood cells or trigger an immune response leading to their destruction.

Autoimmune Disorders

Conditions like autoimmune hemolytic anemia where the immune system mistakenly recognizes red blood cells as foreign and attacks them, causing hemolysis.

Mechanical Trauma

Physical injury or trauma can cause mechanical damage to red blood cells leading to their rupture and hemolysis.

Genetic Disorders

Inherited conditions such as sickle cell disease, thalassemia, and hereditary spherocytosis can lead to abnormal red blood cells that are more prone to hemolysis.[4]

Types of Hemolysis

Intravascular Hemolysis

This occurs within the bloodstream, where red blood cells are destroyed within the vessels. It can result from conditions such as autoimmune hemolytic anemia, transfusion reactions, infections like malaria, or mechanical trauma.

Extravascular Hemolysis

In this type, red blood cells are destroyed outside the bloodstream, primarily in the spleen and liver. Conditions such as hereditary spherocytosis, sickle cell disease, and thalassemia often lead to extravascular hemolysis.

Osmotic Hemolysis

Osmotic hemolysis occurs when red blood cells swell and burst due to exposure to solutions with abnormal osmotic pressure. This can be observed in conditions like hereditary spherocytosis and certain types of anemia.

Immune Hemolysis

Immune-mediated hemolysis occurs when the immune system recognizes red blood cells as foreign and targets them for destruction. This can be caused by autoimmune diseases, alloimmune reactions (such as Rh incompatibility), or drug-induced immune responses.

Mechanical Hemolysis

Mechanical hemolysis occurs due to physical trauma or shear forces that mechanically disrupt red blood cells. This can happen during cardiac valve prostheses, extracorporeal circulation, or when blood passes through narrow vessels or artificial surfaces.[5]

Medication

Corticosteroids

Prednisone and prednisolone are commonly prescribed to suppress the immune system in autoimmune hemolytic anemia, reducing hemolysis by decreasing antibody production.

Immunosuppressive Drugs

Drugs like azathioprine, cyclophosphamide, and rituximab may be used in refractory cases of autoimmune hemolytic anemia where corticosteroids alone are insufficient.

Folic Acid

Folic acid supplementation is often recommended in hemolytic anemias to support red blood cell production and compensate for increased turnover.

Immunoglobulins

Intravenous immunoglobulin (IVIG) therapy may be used in severe cases of autoimmune hemolytic anemia to modulate the immune response and reduce hemolysis.

Eculizumab

Eculizumab, a monoclonal antibody that inhibits the complement system, is used in paroxysmal nocturnal hemoglobinuria (PNH) to reduce hemolysis and prevent thrombosis.

Iron Supplements

Iron supplementation may be necessary in chronic hemolytic anemias to prevent or treat iron deficiency anemia resulting from increased red blood cell turnover.

Blood Transfusions

Transfusions of packed red blood cells may be required to treat severe anemia resulting from hemolysis, especially in acute or life-threatening cases.

Chelating Agents

Chelating agents such as deferoxamine or deferasirox may be used in hemolytic disorders associated with iron overload, such as transfusion-dependent thalassemia.

Hydroxyurea

Hydroxyurea may be used in sickle cell disease to increase fetal hemoglobin levels and reduce the frequency of vaso-occlusive crises and hemolysis.

Supportive Therapy

Supportive measures such as hydration, pain management, and avoiding triggers (e.g., certain medications, infections) are essential in managing hemolysis and associated symptoms.[6]

MATERIAL AND METHODHOLOGY

Plant Profile

Figure No.2: Senegalia Pennata

Synonym

Acacia pennata

Biological source

Senegalia is a genus of flowering plants in the legume family (Fabaceae), and it was formerly classified under the genus Acacia. Acacia pennata, commonly known as climbing wattle or cha-om, is a plant that belongs to the Senegalia genus. It is native to Southeast Asia, including Thailand and other neighboring countries.[7]

Family

Fabaceae

Color

The leaves of Senegalia pennata are typically green, with a slightly lighter shade on the underside. When in bloom, it produces small, fragrant, yellowish-white flowers.

Odour

The odour of Senegalia pennata leaves can vary but is often described as mild and slightly aromatic, typical of many plants in the Acacia genus.

Taste

While taste is not commonly described for Senegalia pennata, some traditional medicinal uses suggest it may have a slightly bitter or astringent taste.[8]

Description

Senegalia pennata is a woody climber or scrambling shrub that can reach heights of up to 5 meters. It has compound leaves with small leaflets arranged alternately along the stem. The plant is armed with sharp thorns along its stems. The flowers are small and clustered, with a pleasant fragrance. The plant often grows in disturbed habitats, along roadsides, and in open forests.[9]

Geographical Distribution:

India

Senegalia pennata is native to India and is widely distributed throughout the country, particularly in regions with tropical and subtropical climates. It is found in states such as Kerala, Karnataka, Tamil Nadu, Maharashtra, and Andhra Pradesh.

Southeast Asia

This species is also found in countries across Southeast Asia, including Thailand, Myanmar, Laos, Vietnam, Cambodia, and Indonesia.

South Asia

Senegalia pennata is present in countries like Sri Lanka, Bangladesh, and Nepal.

East Asia

It can also be found in parts of East Asia, including southern China.

Pacific Islands

Senegalia pennata has been introduced to some Pacific Islands, including Fiji.

Other Regions

Additionally, it may be found in other countries with suitable tropical or subtropical climates and suitable habitat conditions.[10]

Cultivation and Collection

Propagation

Senegalia pennata can be propagated from seeds, which are collected from mature pods. The seeds should be scarified or soaked in hot water to break their hard seed coat before sowing. They can be sown directly into the ground or in seed trays filled with well-draining soil. Germination typically occurs within 1-2 weeks.[11]

Site Selection

Senegalia pennata thrives in sunny locations with well-drained soil. It can tolerate a wide range of soil types, including sandy, loamy, or clayey soils, as long as they are well-draining.

Planting

Transplant seedlings into their permanent growing positions once they have developed several true leaves and are large enough to handle. Space the plants according to their mature size, typically around 1-2 meters apart.[12]

Watering

Newly planted seedlings should be watered regularly to establish a healthy root system. Once established,

Senegalia pennata is relatively drought-tolerant and only requires occasional watering during dry periods.

Pruning

Regular pruning helps promote bushier growth and can control the plant's size. Prune back any dead or damaged branches and shape the plant as desired.

Harvesting

Leaves, young shoots, and tender stems of Senegalia pennata can be harvested for various purposes, including medicinal use and culinary applications. Harvesting should be done carefully to avoid damaging the plant.[13]

Storage

Freshly harvested parts of Senegalia pennata can be used immediately or dried for later use. Store dried leaves and other plant parts in a cool, dry place away from direct sunlight to maintain their quality.[14]

Pest and Disease Management

Senegalia pennata is relatively resistant to pests and diseases. However, occasional pest infestations or diseases such as powdery mildew may occur, which can be managed through cultural practices or the use of organic pesticides.

Chemical constituent

Bioactive compounds: Alkaloids, flavonoids, glycosides, phenols, phytosterols, terpenoids, and saponins

Chemicals

Oleic acid, ferulic acid, myristic acid, palmitic acid, quercetin 3-O-(4′-O-acetyl)-rhamnopyranoside, p-coumaroyl-glucoside, p-coumaroylquinic acid, and steroidal sapogenin.[15]

USES

The dried and powdered berry can help reduce blood pressure. The young leaves can be harvested in the spring through summer and are a good source of vitamin A, calcium, phosphorus, vitamin C, iron, fiber, and B vitamins. The leaves can be consumed raw, but the offensive odor of the fresh leaves is generally off-putting. They are most commonly cooked to reduce the smell.[16]

Preparation of plant extracts senegalia penneata

Collection of Plant Material

Harvest fresh leaves, young shoots, or other plant parts of Senegalia pennata during the appropriate season as mentioned earlier.

Cleaning and Drying

Clean the collected plant material to remove any dirt or debris. Allow the plant material to air dry in a well-ventilated area away from direct sunlight until it is thoroughly dried. This step helps to prevent the growth of mold and bacteria during storage.

Grinding or Crushing

Once dried, grind or crush the plant material into smaller pieces using a mortar and pestle or a grinder. This increases the surface area of the plant material, facilitating the extraction proces.[17]

Preparation of Ethanolic Extract

The extraction preparation procedures differed slightly from those detailed in. The leaf sample wasowashed with ordinary water, alleshtly from those detailed in The blender to be ground into powder. Various ratios ored to dry, and then put into for the Soxhlet extraction procedure. 6 to 8 hours after thanol are used as gathered Utilise a muslin cloth to filter it. Centrifuge the far the extract has been gathered 15 minutes at 4,000 rpm and 25 °C. After being gathered the supernatant was retained for drying.[18]

Preliminary phytochemical screening of Extraction

Preparation of phosphate buffer solution

To prepare a phosphate buffer solution, you will need to mix solutions of monobasic sodium phosphate (NaH2PO4) and dibasic sodium phosphate (Na2HPO4) in appropriate proportions to achieve the desired pH. Here's a general procedure for preparing a phosphate buffer solution:[19]

Calculate the Proportions

Determine the desired pH of the buffer solution using a buffer calculator or table. Then, calculate the appropriate proportions of monobasic and dibasic sodium phosphate solutions needed to achieve the desired pH. The Henderson-Hasselbalch equation can be used for this purpose.

Prepare Stock Solutions

Prepare stock solutions of monobasic sodium phosphate (0.2 M) and dibasic sodium phosphate (0.2 M) by dissolving the appropriate amounts of each salt in distilled water. Ensure that the salts are completely dissolved.[20]

Mixing

Slowly add the calculated volumes of the monobasic and dibasic sodium phosphate stock solutions to a clean glass container, while stirring continuously. Continue mixing until the desired pH is reached.

Adjust pH if Necessary

Measure the pH of the buffer solution using a pH meter or pH indicator paper. If the pH is not within the desired range, adjust it by adding small amounts of either monobasic or dibasic sodium phosphate solution as needed, and then recheck the pH.[21]

Final Dilution (if needed)

Once the pH is adjusted to the desired range, adjust the volume of the buffer solution to the final desired volume by adding distilled water if necessary. Mix thoroughly.

Filtering (optional)

If desired, filter the buffer solution using a sterile filter to remove any particulate matter or impurities.[22]

Sterilization (optional)

If the buffer solution is to be used for biological applications, it may be sterilized by autoclaving or filtering through a sterile membrane filter.

Storage

Store the prepared phosphate buffer solution in a clean, sterile container with an airtight lid. It can be stored at room temperature for short-term use or refrigerated for longer-term storage.

The process for making erythrocyte cells

Blood samples were collected from healthy volunteer donors of blood. A sterile saline phosphate buffer solution (PBS Buffer) 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. [23]

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.[24]

Phytochemical Investigation:[25]

Sr No.	Name of Test	Observation	Inference
1	Test For Phenol: Extract Mixed with 2 ml of 2% of Solution of $Fecl_3$	Blue/Green Colour	Phenol present
2	Test for Saponin: The Extract was taken in test tube and shaken vigorously with water	Formation of stable foam	Saponin present
3	Test for Tannins: Extract Mixed with 2% of $Fecl_3$	No Black Colour	Tannin absent
4	Test For Terpenoids: The Extract mixed with chloroform. then 2ml of conc. sulphuric acid was added carefully and shaken gently	Reddish brown colour observed in the interphase	Terpenoids Present
5	Test for flavonoids: Extract was treated with few drops of sodium hydroxide solution	Formation of intense yellow colour.which becomes colourless on the addition of dilute acid.	Flavonoids present
6	Test for glycosides: The extract was mixed with 2ml of glacial acetic acid containing few drops of 2% $Fecl_3$; mixture poured into another tube containing 2ml of conc. Sulphuric acid.	A brown ring at the inerphase	Carbohydrate absent
7	Test for protein: The extract treated with few drops of conc. Nitric acid	Formation of yellow colour.	Protein absent
8	Test for alkaloids: Dragendroff's test: To a few ml of filtrate ,1 or 2ml of Dragendorff reagent Mayer's test: To few ml of extract ,2drops of mayor's reagents. Hager's test: To few ml of extract 1or2ml of hanger's reagent (saturated solution of picric acid) were added Wagner's 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 senegalia pennata extract.

EXPERIMENTAL WORK

Procedure

Preparation of Plant Extracts Senegalia pennata

The extraction preparation procedures differed slightly from those detailed in. The leaf sample was washed with ordinary water, alleshtly from those detailed in the blender to be ground into powder. Various ratios ored to dry, and then put into for the Soxhlet extraction procedure. 6 to 8 hours after ethanol are used as gathered Utilize a muslin cloth to filter it. Centrifuge the far the extract has been gathered 15 minutes at 4,000 rpm and 25 °C. After being gathered the supernatant was retained for drying. [26]

Preliminary phytochemical screening of Extraction

Preparation of Phosphate Buffer Solution

To prepare a phosphate buffer solution, you'll need to mix monobasic sodium phosphate (NaH_2PO_4) and dibasic sodium phosphate (Na_2HPO_4) in appropriate proportions to achieve the desired pH. Here's a general procedure: [27]

Calculate Buffer Ratio

Use the Henderson-Hasselbalch equation to calculate the ratio of monobasic to dibasic sodium phosphate needed to achieve the desired pH. The equation is $pH = pKa + \log([A^-]/[HA])$, where $[A^-]$ is the concentration of the conjugate base and [HA] is the concentration of the weak acid.

Prepare Stock Solutions

Prepare separate stock solutions of monobasic sodium phosphate (0.1 M) and dibasic sodium phosphate (0.2 M) by dissolving the appropriate amounts of each salt in distilled water. Ensure complete dissolution.

Mixing

Mix the stock solutions together in the calculated ratio to achieve the desired pH. For example, to prepare a pH 7.4 buffer, mix 100 ml of 0.1 M monobasic sodium phosphate with 400 ml of 0.2 M dibasic sodium phosphate.

Adjust pH (if necessary)

Measure the pH of the buffer solution using a pH meter or pH indicator strips. If the pH is not within the desired range, adjust it by adding small amounts of either monobasic or dibasic sodium phosphate solution as needed, and then recheck the pH.

Final Dilution (if needed)

Once the pH is adjusted to the desired range, adjust the volume of the buffer solution to the final desired volume by adding distilled water if necessary. Mix thoroughly.

Filtering (optional)

If desired, filter the buffer solution using a sterile filter to remove any particulate matter or impurities.

Sterilization (optional)

If the buffer solution is to be used for biological applications, it may be sterilized by autoclaving or filtering through a sterile membrane filter.[28]

Storage

Store the prepared phosphate buffer solution in a clean, sterile container with an airtight lid. It can be stored at room temperature for short-term use or refrigerated for longer-term storage.

Preparation of Erythrocyte Cell

Preparation of erythrocytic cells, also known as red blood cells, involves isolation from whole blood. Here's a general procedure:[29]

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.

Washing

Wash the erythrocytic cell pellet multiple times with an isotonic buffer solution (e.g., phosphate-buffered saline, PBS) to remove any remaining plasma proteins and platelets. 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

Store the prepared erythrocytic cell suspension in aliquots at appropriate temperatures. Erythrocytes are typically stored at 4°C for short-term use or frozen at -20°C or -80°C for long-term storage. Avoid repeated freeze-thaw cycles to maintain cell integrity.

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

Wash the RBC pellet multiple times with an isotonic buffer solution (e.g., phosphate-buffered saline, PBS) to remove any residual plasma proteins and platelets. Centrifuge the RBCs after each wash and carefully remove the 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.[30]

Incubation with RBCs

Incubate the RBC suspension with the test samples at physiological conditions (e.g., 37°C, pH 7.4) for a specific period, typically 1-2 hours.

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.[31]

Calculation of Hemolytic Activity

Calculate the percentage of hemolysis using the formula: Hemolysis (%) = (Absorbance of test sample - Absorbance of negative control) / (Absorbance of positive control - Absorbance of negative control) × 100[32]

RESULT

The Following Formula used to determine the proportion of Hemolysis:

$\%\ \text{Hemolysis} = [(At-An) / (Ac-An)] \times 100$

Where,

At: absorbance of the test sample

An: minimal control absorbance (phosphate buffered saline solution PBS)

Ac: maximum control absorbance (distilled water)

Observation

Sr. No	Concentration	Absorbance	Hemolysis %	Protection
1	25ml	0.024	88.05%	0.08
2	50ml	0.124	38.30%	0.38
3	75ml	0.191	4.97%	0.04
4	100ml	0.350	4.12%	-0.74

Table No.2: Absorbance of sample

Phytochemical screening is a useful technique for identifying bioactive compounds utilized in drug synthesis and for determining the molecular makeup of different plant extracts. Results of phytochemical testing on ethanolic extracts of the leaves and stem of Senegalia penneata Screening of Senegalia penneata indicated the presence of moisture, elemental components like carbon, hydrogen, nitrogen, and sulfur, but not reducing sugar. The plant's medicinal potential is demonstrated by the presence of these compounds. Since there is no reducing sugar in the plant's stem or leaf, tests can be performed to determine the different phenolic compounds, amino acids, and therapeutic value of the plant.

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 Senegalia pennata

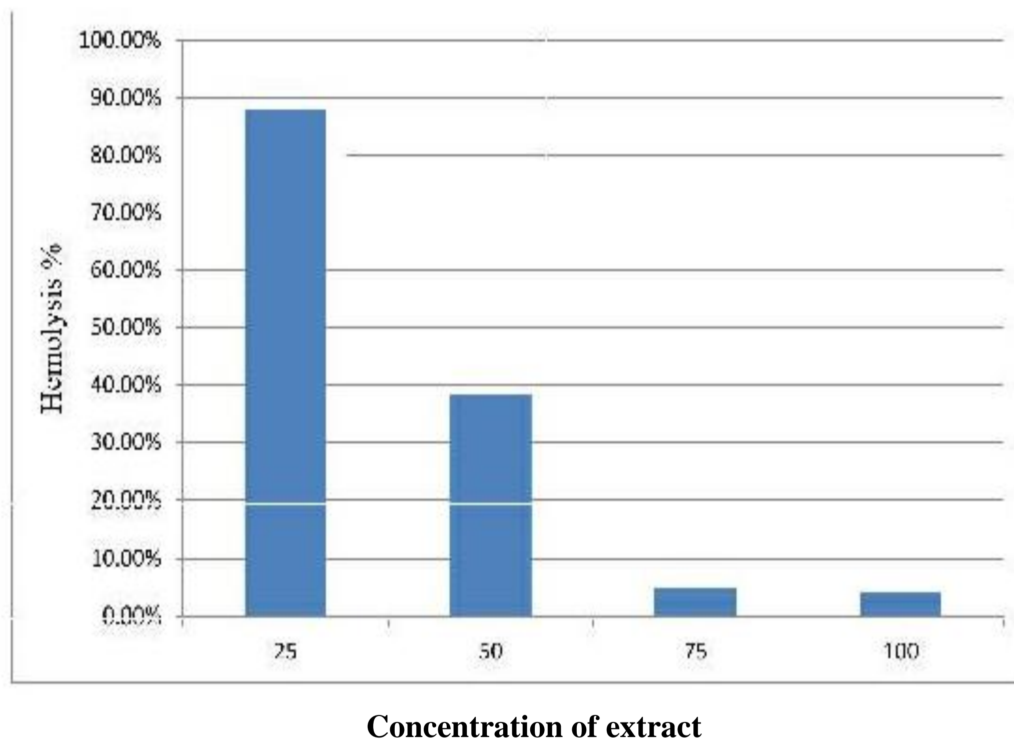
(-) indicates the absence of Compound

(+) indicates the presence of Compound

The ability of the ethanolic extract of Senegalia penneata to hemolyze was evaluated using erythrocytes, and the results were expressed as a percentage of hemolysis. The results (table no.4) showed that the extracts under investigation had an effect on hemolysis. The ethanolic extract causes the least amount of hemolytic activity when it is concentrated at 100 g/ml; concentrations of 75 g/ml, 4.97%, 50 g/ml, and 38.30% cause the least amount of hemolytic activity, while 25 g/ml, or 88.05%, causes the most. The results additionally indicated that the degree of hemolysis is dependent on the extract concentration. The following categories can be used to group the hemolytic effects of the different test extracts: Value range: 25 g/ml to 100 g/ml, with 50 g/ml to 75 g/ml. The phytochemical components found in medicinal plants, such as flavonoids, phenol, saponin, and glycosides, are abundant and have specific pharmacological effects on human body. Among others, terpenoids and alkaloids. Hemolytic activity of any substance indicates that it is generally cytotoxic to normal, healthy cells. The medium hemolytic response of the four extracts indicates their medium cytotoxicity towards human erythrocytes impact.
This assay helpful in identifying whether or not cytotoxic action in linked to actual membrane damage.

Sr No	Concentration	Hemolysis%
1	25	88.05%
2	50	38.30%
3	75	4.97%
4	100	4.12%

Table No.4: Percentage of Hemolysis



Concentration of extract
Graph No.1: Graphical Presentation of Hemolysis

DISCUSSION

The assessment of in-vitro hemolytic activity of Senegalia pennata presents an intriguing avenue for understanding the potential biological effects of this plant extract on red blood cells. Hemolysis, the rupture or destruction of red blood cells, can occur due to various factors including chemical agents, toxins, or natural compounds. Senegalia pennata, also known as the climbing wattle, possesses a diverse array of phytochemicals, making it a subject of interest for biomedical research.

Through in-vitro studies, researchers can elucidate the hemolytic properties of Senegalia pennata extract by exposing red blood cells to different concentrations of the plant extract and assessing the degree of hemolysis. This process allows for the determination of the extract's cytotoxic effects on erythrocytes, potentially shedding light on its safety profile or therapeutic potential.

The discussion surrounding the research on Senegalia pennata's hemolytic activity necessitates consideration of several key points. Firstly, the concentration-dependent nature of hemolysis should be evaluated to ascertain any dose-response relationships. Additionally, the identification of specific phytochemical constituents responsible for the observed hemolytic effects is crucial for understanding the underlying mechanisms.

Furthermore, the implications of the findings on human health and pharmacological applications should be addressed. While hemolysis may indicate cytotoxicity, it could also signify potential therapeutic benefits such as antimicrobial or anticancer properties. Therefore, a comprehensive discussion should encompass both the risks and benefits associated with Senegalia pennata extract.

CONCLUSION

The in-vitro assessment of the hemolytic activity of Senegalia pennata extract revealed a concentration-dependent effect on erythrocytes. At lower concentrations, the extract exhibited minimal hemolysis, indicating its potential safety at these levels. However, at higher concentrations, a significant increase in hemolytic activity was observed, suggesting a cytotoxic effect. These findings highlight the importance of dosage in the therapeutic application of S. pennata, as excessive amounts could lead to hemolytic side effects. The study underscores the need for further investigation into the specific bioactive compounds responsible for this activity and their mechanisms of action. Additionally, in vivo studies are essential to better understand the safety profile and therapeutic potential of S. pennata. Overall, while S. pennata shows promise as a medicinal plant, careful consideration of its hemolytic properties is crucial for its safe use in clinical settings.

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